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Announcement/Avis

Journal Title to Change
January 1980

Effective with the January 1980 issue, the name of the ***Journal*** will be changed to the ***Canadian Journal of Fisheries and Aquatic Sciences***. Numbering of the Journal will begin at Volume 37, Number 1. This change resulted from a Canadian Government Bill which repealed the Fisheries Research Board Act.

The title of the other series of our publications will be changed as follows:

— *Bulletin of the Fisheries Research Board of Canada* to ***Canadian Bulletin of Fisheries and Aquatic Sciences.***

— *Miscellaneous Special Publication* to ***Canadian Special Publication of Fisheries and Aquatic Sciences.***

— *Technical Report* to ***Canadian Technical Report of Fisheries and Aquatic Sciences.***

— *Manuscript Report* to ***Canadian Manuscript Report of Fisheries and Aquatic Sciences.***

— *Industry Report* to ***Canadian Industry Report of Fisheries and Aquatic Sciences.***

— *Data Report* to ***Canadian Data Report of Fisheries and Aquatic Sciences.***

— *Translation series* to ***Canadian Translations of Fisheries and Aquatic Sciences.***

Modification de titre du *Journal*
en janvier 1980

À compter du numéro de janvier 1980, soit le n° 1, volume 37, le titre du *Journal* deviendra le ***Journal canadien des sciences halieutiques et aquatiques***. Cette modification fait suite à la présentation d'un projet de loi du gouvernement canadien qui a annulé la Loi sur le Conseil des recherches sur les pêcheries.

Les titres des autres publications seront aussi modifiés comme suit:

— Le *Bulletin de l'Office des recherches sur les pêcheries du Canada* devient ***Bulletin canadien des sciences halieutiques et aquatiques.***

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— Le *Rapport manuscrit* devient ***Rapport manuscrit canadien des sciences halieutiques et aquatiques.***

— Le *Rapport à l'industrie* devient ***Rapport canadien a l'industrie sur les sciences halieutiques et aquatiques.***

— Le *Rapport statistique* devient ***Rapport statistique canadien des sciences halieutiques et aquatiques.***

— La *série de traductions* devient ***Traductions canadiennes en sciences halieutiques et aquatiques.***

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Relationship of Cadmium-Induced Hypocalcemia with Mortality in Rainbow Trout (*Salmo gairdneri*) and the Influence of Temperature on Toxicity

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ROCH, M., AND E. J. MALY. 1979. Relationship of cadmium-induced hypocalcemia with mortality in rainbow trout (*Salmo gairdneri*) and the influence of temperature on toxicity. J. Fish. Res. Board Can. 36: 1297–1303.

Cold-acclimated (6°C) rainbow trout, exposed to lethal concentrations of cadmium, survived longer than warm-acclimated (12 and 18°C) trout. Ten-day lethal thresholds indicated that cold-acclimated trout could also resist greater concentrations of cadmium. Calcium concentrations in the plasma of fish, exposed to 0.3 mg Cd/L, declined steadily during the period of exposure when subjected to five thermal regimes, different combinations of acclimation and experimental temperatures. The rate of decline in calcium concentrations was correlated with the rate of mortality. Survival times of 12°-acclimated trout subsequently exposed to cadmium at 6, 12, and 18°C were inversely dependent on temperature, whereas survival times of trout acclimated to 6, 12, and 18°C and subsequently exposed to cadmium at acclimation temperatures do not show the same temperature dependence. Survival times of 12 and 18°-acclimated and exposed fish were similar, indicating that temperature compensation may promote longer resistance of 18°-acclimated and exposed fish than when they are acclimated to 12°C and abruptly transferred and exposed to cadmium at 18°C. Symptoms of cadmium poisoning of the fish are consistent with the effects of extreme hypocalcemia and are similar to those reported in the literature for various fish. The decline of calcium concentrations in the plasma may be the direct cause of mortality.

Key words: Cadmium, temperature, hypocalcemia, rainbow trout, *Salmo gairdneri*, toxicity

ROCH, M., AND E. J. MALY. 1979. Relationship of cadmium-induced hypocalcemia with mortality in rainbow trout (*Salmo gairdneri*) and the influence of temperature on toxicity. J. Fish. Res. Board Can. 36: 1297–1303.

Des truites arc-en-ciel acclimatées au froid (6°C) survivent plus longtemps à une exposition à des concentrations létales de cadmium que des truites acclimatées à la chaleur (12 et 18°C). Les seuils létaux de 10 jours indiquent que les truites acclimatées au froid pourraient également résister à de plus fortes concentrations de cadmium. Les concentrations de calcium dans le plasma des poissons exposés à 0,3 mg Cd/L diminuent régulièrement durant l'exposition à

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cinq régimes thermiques et à différentes combinaisons d'acclimatations et de températures expérimentales. Il y a corrélation entre la vitesse de diminution des concentrations de calcium et le taux de mortalité. Les temps de survie de truites acclimatées à 12°C et exposées ensuite au cadmium à 6, 12 et 18°C sont inversement dépendants de la température, alors que les temps de survie de truites acclimatées à 6, 12 et 18°C et exposées ensuite au cadmium aux températures d'acclimatation ne montrent pas cette dépendance de la température. Les temps de survie des poissons acclimatés à 12 et 18°C et exposés sont semblables, signes que la compensation de température peut favoriser une plus longue résistance des poissons acclimatés à 18°C et exposés que celle de poissons acclimatés à 12°C et transférés abruptement et exposés au cadmium à 18°C. Les symptômes d'empoisonnement par cadmium des poissons sont conformes aux effets d'une hypocalcémie extrême et sont identiques aux données publiées pour divers poissons. La diminution des concentrations de calcium dans le plasma peut être la cause directe de mortalités.

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TEMPERATURE is an important controlling factor of metabolism in poikilotherms and can have a marked effect on the toxicity of heavy metals to fish (MacLeod and Pessah 1973; Hodson and Sprague 1975).

Cadmium contamination in North American waters and its highly toxic effects on aquatic organisms have been extensively reviewed (Page and Bingham 1973; EPA 1976; EIFAC 1978). Little is known of the influence of temperature on cadmium toxicity to freshwater fish and since the temperature of Canadian waters varies a great deal with season and latitude, we designed an experiment to determine how temperature alters cadmium toxicity to rainbow trout. We hypothesize that cadmium toxicity may vary considerably, dependent upon thermal regimes of water.

Although a number of studies have shown physiological, biochemical and hematological disturbances following the exposure of fish to cadmium (Gardner and Yevich 1970; Larsson et al. 1976), no correlation of mortality with these disturbances has been demonstrated. Since a number of studies of cadmium toxicity to fish have indicated skeletal abnormalities (Bengtsson et al. 1975; Eaton 1974) and one has shown an effect on calcium ion regulation (Larsson et al. 1976) we were interested in noting whether changes in calcium concentrations in the plasma, during a period of acute exposure, could be correlated with mortality at different temperatures. Magnesium concentrations were also measured because magnesium and calcium ions are associated in a variety of biological membranes. Plasma osmolalities were determined to see whether any changes in the divalent cation concentrations were due to changes in plasma water content.

The results presented in this paper suggest that hypocalcemia in rainbow trout exposed to cadmium is a major factor affecting mortality and that cadmium toxicity is affected to a considerable extent by temperature.

Materials and Methods

FISH AND WATER CHARACTERISTICS

Juvenile rainbow trout (*Salmo gairdneri*) obtained from La Pisciculture Mont Sutton at Mont Sutton, Quebec, were maintained in holding tanks at 11°C. The dechlorinated City of Montreal water had a hardness of 125 mg/L as $CaCO_3$, alkalinity was 90 mg/L as $CaCO_3$, and the pH ranged from 7.8 to 7.9. Oxygen concentrations were measured daily and were greater than 85% saturation during all experiments. Chemical analyses were performed according to APHA et al. (1971) methods. All acclimation test and temperatures were maintained within 0.5°C of 6, 12, or 18°C.

Fish were fed daily with trout chow (Ewos-2% of body weight per day) during holding and acclimation. The average weight of the fish was 9.2 ± 0.6 g in the first experiment (Dec. 1977) and 20.0 ± 1.0 g in the second experiment (June 1978); mean fork lengths were 9.3 and 12.6 cm, respectively.

ACCLIMATION

The trout were acclimated to 6, 12, or 18°C. The temperature was changed from an initial temperature of 11°C at the rate of one degree per day and the fish were considered thermally acclimated after maintenance at either 6, 12, or 18°C for 14 d. A period of 2 wk was deemed adequate since Peterson and Anderson (1969) indicate that the metabolic rate of Atlantic salmon (*Salmo salar*) stabilizes within 2 wk following a temperature change from 18° to 6°C or from 6° to 18°C. Photoperiod during acclimation and test periods was 12 h light/12 h dark.

EXPOSURE APPARATUS

The experimental flow-through apparatus consisted of twenty four 90-L polyethylene tanks and three head boxes which supplied water at 6, 12, or 18°C. Each head box supplied eight tanks with water at the same temperature. Flow rates were regulated with flow meters (Manostat Corp. New York, N.Y.) at 750 mL/min, giving a 95% replacement time of 6 h and >5 L of water per gram of fish per day (see Sprague 1973).

Assayed reagent grade $CdCl_2 \cdot 2\frac{1}{2}H_2O$ was dissolved in dechlorinated water acidified to pH 6 with sulphuric acid and the cadmium solution was delivered from 20-L Mariotte bottles at a constant rate of 1.5 mL/min (see Leduc 1966) to provide the desired test concentration after 500-fold dilution.

Samples of cadmium concentrations in the test tanks were taken daily and analyzed by flame atomic absorption

spectrophotometry (Perkin–Elmer Model 503) and by graphite furnace equipped with a deuterium background corrector for nominal concentrations of 30 μg Cd/L and less. All concentrations reported refer to assayed total cadmium as Cd^{2+}.

Experimental Procedure

For the first experiment, 10 rainbow trout were randomly assigned to each of seven test tanks 2 d before exposure to a nominal logarithmic series of cadmium concentrations ranging from 0.01 to 10.0 mg Cd/L. The fish were exposed to cadmium at their respective temperatures of acclimation and observations of mortality were carried out for 10 d according to the recommendations of Sprague (1973) for acute bioassays. Median effective time, slope of percent mortality versus time, and their 95% confidence limits were calculated according to Litchfield (1949).

For the second experiment, 40 fish were acclimated to 6, 12, and 18°C and the fish from each temperature acclimation group were randomly assigned among four tanks (10 per tank) at their respective acclimation temperatures. Of these, one subgroup of 10 was not exposed to cadmium and was regarded as 0 h exposed; the other three subgroups of 10 were exposed to 0.3 mg Cd/L for 48, 96, and 168 h at 6°C and for 24, 48, and 60 h at both 12° and 18°C. The trout were exposed for these lengths of time because they represent intervals within the limit indicated by the median survival times determined from experiment one which were 170, 85, and 78 h at 6, 12, and 18°C, respectively. The concentration of 0.3 mg Cd/L was chosen as a lethal concentration from the flat region of the mortality curve in experiment one, so that small deviations of cadmium concentrations among temperature treatments would not have a pronounced effect on survival time.

An additional 80 fish were acclimated at 12°C. Forty of these were abruptly transferred to four tanks (10 per tank) at 6°C and 40 were distributed among four tanks (10 per tank) at 18°C. These abruptly transferred fish were not considered acclimated to the test temperature and are intended to simulate sudden increases and decreases in temperature. One control tank at each temperature did not receive cadmium. The other three groups of 10 fish at each temperature were exposed to 0.3 mg Cd/L beginning at 24 h after transfer for 48, 96, and 168 h at 6°C, and for 24 and 30 h at 18°C. The experiment was terminated for the 18°C-exposed (12°-acclimated) group when 50% mortality occurred at 30 h (maximum error — 2 h) and for the 6°C-exposed (12°C-acclimated) group at 168 h when 50% mortality was observed (maximum error — 10 h). Median survival times (Fig. 3) for fish exposed to 0.3 mg Cd/L at the temperature of acclimation were determined from regression equations calculated from ET50's in the concentration range of 0.03–3.29 mg Cd/L at 18°C, 0.11–3.11 mg Cd/L at 12°C, and 0.10–3.80 mg Cd/L at 6°C in experiment one.

Blood samples from 5 to 10 fish were collected by caudal severance into heparinized test tubes and pooled according to thermal regime and period of exposure. Quantities of plasma (100 μL) were diluted to 5 mL with 0.1% (as La) lanthanum solution and calcium and magnesium concentrations of three replicates were determined by flame atomic absorption (Perkin–Elmer Model 503). Plasma osmolality was determined by freezing point depression using a techican autoanalyzer.

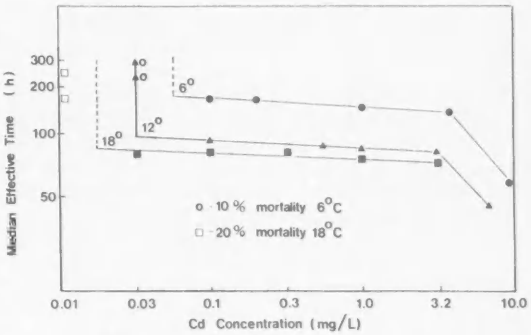

FIG. 1. Mortality times of juvenile rainbow trout exposed to cadmium and acclimated at 6, 12, and 18°C. Dashed lines represent approximate 10-d lethal thresholds (50% mortality) at 6 and 18°C.

Results

Experiment 1

Figure 1 shows the effect of temperature on the median survival time (ET50) of rainbow trout exposed to cadmium. It is apparent that fish acclimated and exposed at 6°C survived longer than those acclimated and exposed at 12°C, which in turn survived longer than fish at 18°C.

Regression equations were calculated from the ET50's in the range of 0.1–3.8 mg Cd/L which is the common range for all three temperatures that caused 50% mortality. The slopes of these equations were not significantly different from 0 at all three temperatures ($P < 0.05$). Median survival times calculated from the regression equations at 18°C are significantly less ($P < 0.10$) than at 12° for all concentrations between 0.10 and 3.2 mg Cd/L and ET50's at 12°C are significantly less than at 6°C ($P < 0.01$) in the same concentrations range.

Ten day lethal threshold concentration was between 10 and 30 μg Cd/L at 18°C; at 12°C it was 30 μg Cd/L and at 6°C it was between 30 and 100 μg Cd/L. Lethal thresholds increased with decreasing temperature.

Symptoms of poisoning at all Cd concentrations tested included hypersensitivity, erratic and violent swimming followed by lethargy and loss of equilibrium. On several occasions, fish that were taken out of the tanks showed no response to handling nor opercular movement but exhibited rhythmic but uncoordinated movements of the body musculature and tetanic extensions of the jaw.

Experiment 2

Figure 2 shows the decline in plasma calcium concentrations with time of exposure to 0.3 mg Cd/L all temperatures. In all cases some mortality had occurred when calcium concentrations of the plasma were below 30 meq/L, so the time of calcium reduction to 3.0

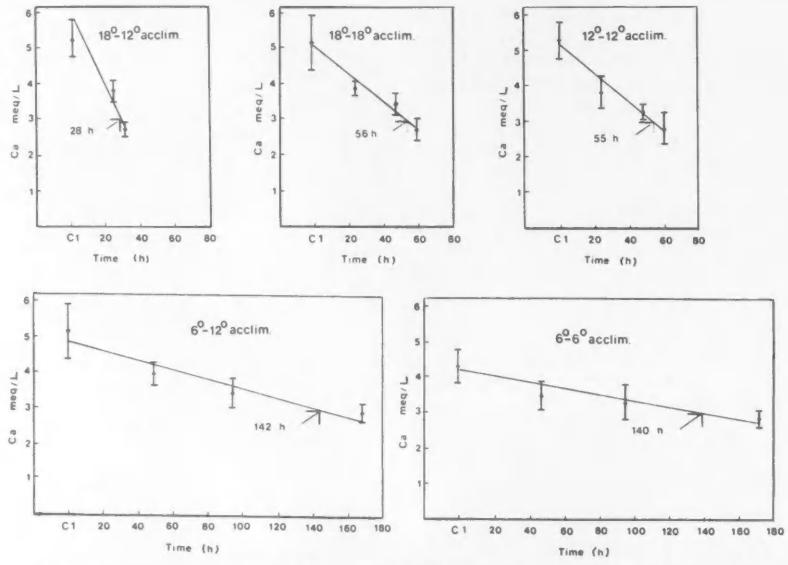

FIG. 2. Decline of calcium concentrations in the plasma of rainbow trout with time of exposure to 0.3 mg Cd/L and the effect of acclimation and exposure temperature. Bars represent 95% confidence limits of the means. Controls (Cl) represent 0 h of exposure.

meq/L (calcium reduction time) was used as a basis for comparison with survival time under different temperature regimes. The calcium reduction times were determined by a regression equation and 95% confidence limits of this time were calculated according to Sokal and Rohlf (1969, p. 446).

Magnesium concentrations (Table 1) in the plasma of 12 and 18°C exposed fish increased during the ex-

TABLE 1. Change of magnesium and osmolality concentrations of plasma in rainbow trout with time of exposure to 0.3 mg Cd/L and the variation with temperature of acclimation and exposure.

Temperature E-exposure A-acclimation	Cd concentration (mg/L) Mean ± SD	Time of exposure (hours)	Mean ± 95% confidence limits of Mg in plasma (meq/L)	Plasma osmolality (m osm/L) Mean ± SD
		0	2.14 (0.27)	301 (9.6)
E-18°C	0.28 (0.04)	24	3.81 (0.28)	291 (5.6)
A-12°C		30	3.39 (0.09)	299 (15.0)
		0	2.29 (0.14)	295 (4.4)
E-18°C	0.33 (0.02)	24	2.93 (0.21)	303 (2.9)
A-18°C		48	3.72 (0.17)	303 (4.7)
		60	3.95 (0.12)	294 (4.0)
		0	2.13 (0.05)	289 (5.5)
E-12°C	0.30 (0.04)	24	2.86 (0.08)	300 (10.8)
A-12°C		48	2.61 (0.13)	287 (8.7)
		60	2.78 (0.10)	294 (8.0)
		0	2.23 (0.16)	297 (4.7)
E-6°C	0.31 (0.02)	48	1.92 (0.10)	288 (6.4)
A-12°C		96	2.34 (0.06)	294 (4.0)
		168	2.31 (0.07)	301 (4.0)
		0	1.92 (0.19)	291 (5.6)
E-6°C	0.34 (0.02)	48	1.84 (0.17)	301 (4.0)
A-6°C		96	2.34 (0.06)	302 (4.5)
		168	2.46 (0.24)	296 (3.6)

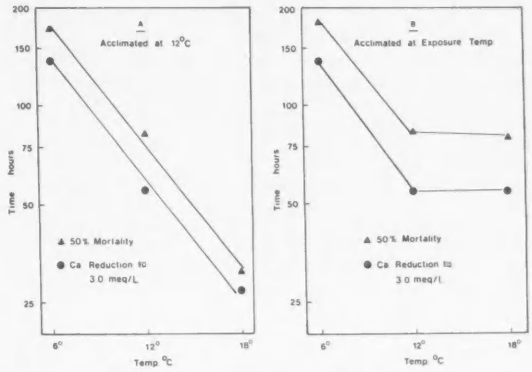

FIG. 3. Time to 50% mortality and calcium reduction time of rainbow trout exposed to 0.3 mg Cd/L at 6, 12, and 18°C. A, Acclimated to 12°C; B, Acclimated to the temperature of exposure to cadmium.

posure period, reached a maximum before the final sampling time and in some cases declined again. Increases at 6°C were minimal and changes in magnesium concentrations of the plasma show no definite trend with increasing length of exposure to cadmium for all temperature regimes.

Plasma osmolality (Table 1) was determined for all blood samples and despite the changes in calcium and magnesium concentrations, no effect of cadmium exposure on osmolality is evident. Analysis of variance indicates no significant difference among treatments ($P < 0.05$).

Figure 3 compares survival times and calcium reduction times for the five thermal test regimes. Fish acclimated to 12°C and exposed to cadmium at 6, 12, and 18°C show that survival time depends inversely on temperature. The Q_{10} of the rate of mortality (1/ET50) is 3.98. Calcium reduction times show the same inverse temperature dependence and closely parallel the survival times. In contrast fish acclimated and exposed at 18°C, exhibited a marked deviation from temperature dependence in terms of both survival and calcium reduction time (Fig. 3). Both parameters are approximately double those of 12°-acclimated fish exposed to cadmium at 18°C. The difference in calcium reduction and survival times between 18° and 12°-acclimated fish is much less than that between 12° and 6°-acclimated fish.

Discussion

The results indicate that temperature has a marked effect on the toxicity of cadmium to rainbow trout. Cold-acclimated (6°C) fish showed a greater ten day lethal threshold concentration and survived approximately twice as long as warm-acclimated (18°C) trout, that were exposed to the same concentrations. Roberts (1973) indicates that gill ventilation rate increases with an increase in temperature; this may cause more rapid exposure to cadmium and decreased survival time.

The flat mortality curves in the concentration range of 0.1–3.8 mg Cd/L at all three temperatures in experiment 1 resemble the cadmium toxicity curve reported by Ball (1967) with rainbow trout. Such an effect could be partly accounted for by a biochemical process, such as binding to thioneins, which detoxifies greater quantities of cadmium by sequestering it in proportion to concentrations of cadmium in the water.

Olson et al. (1978) indicate that rainbow trout gill, liver and kidney contain metallothionein, which has a high affinity for heavy metals and is believed to be involved in their detoxification. Cadmium has been shown to induce the synthesis of metallothionein in rats (Hidalgo et al. 1976) and Olafson and Thompson (1974) showed an increased quantity of metallothionein in the liver of the copper rockfish (*Sebastes caurinus*) as the cadmium dose increased. Increased detoxification of cadmium by increased thionein synthesis and binding to metallothioneins at higher cadmium concentrations may be related to the flat mortality curve that was observed.

Fish acclimated to the test temperatures showed a marked deviation from temperature dependence at 18°C, but the rate of mortality (1/ET50) of 12°-acclimated fish exposed to cadmium at 6, 12, and 18°C was temperature dependent, suggesting that the rate at which cadmium affects the fish is governed by temperature in a way that resembles the effect of temperature on metabolic rate following rapid temperature change.

Acclimation of fish to different temperatures often involves qualitative enzyme changes and alterations of metabolic pathways resulting in temperature compensation (Hochachka and Somero 1973). For example, Roberts (1973) indicates that the Q_{10} of routine metabolic rate of thermally acclimated pumpkinseed sunfish (*Lepomis gibbosus*) is 1 between 10 and 17.5°C. Such temperature compensation of the whole organism or of a target organ or enzyme might account for the slight decrease in survival times between 12 and 18°C-acclimated fish as compared to the much greater difference in survival times between 6 and 12°C-acclimated fish.

The steady decline of calcium concentrations in the plasma at all five temperature combinations cannot be due to changes in plasma water content since plasma osmolality showed no significant variation. The extreme hypocalcemia can severely impair nerve and muscle function, and the symptoms of acute poisoning, of hypersensitivity, rhythmic muscle contractions and tetany are common symptoms of hypocalcemia in humans (Wintrobe et al. 1974, p. 1944). Similar symptoms of acute cadmium poisoning in fish have been reported by a number of researchers (Cearley and Coleman 1974; Benoit et al. 1976; Pickering and Gast 1972; Pascoe and Matthey 1977). The similarity of symptoms suggests that hypocalcemia may be a com-

mon response of acute exposure of fish to cadmium.

The cause of hypocalcemia was not determined in this experiment. It may result from interference with calcium transport systems in the gill or impaired resorptive ability of the kidney tubules. Impaired renal function is a common effect of acute and chronic cadmium exposure in mammals (Nordberg 1974) and renal tubule alterations were also reported by Gardner and Yevich (1970) in the euryhaline killifish (*Fundulus heteroclitus*) after exposure to cadmium. Damage to renal tubules could lead to excessive loss of calcium in the urine of freshwater fish and subsequent hypocalcemia. Saris and Jarvisalo (1977) demonstrated that 1 μmol Cd/L inhibited calcium ion transport by rat kidney mitochondria. Interference with active calcium transport in the gills or kidneys of rainbow trout might lead to hypocalcemia.

Kennedy (1966) reported a fall in serum calcium levels in rabbits injected subcutaneously with cadmium and suggested that the decrease was due to increased renal excretion. Friberg et al. (1971) report serum calcium levels in the low end of the normal range in patients that had been exposed to cadmium contamination and Larson et al. (1976) report hypocalcemia in flounder exposed to cadmium. The frequency of reports implicating disturbances in calcium ion regulation with cadmium poisoning suggests that cadmium's detrimental effects may be manifested primarily as a disturbance in calcium regulation.

Pronounced hypermagnesemia was also observed in Kennedy's (1966) experiment but did not occur at all thermal regimes. Larson et al. (1976) also report hypermagnesemia in flounder exposed to cadmium. No significant correlation between the rate of mortality and the rate of magnesium increase is evident in our study and we believe that the increase in magnesium levels does not account for mortality.

For all five thermal regimes calcium reduction time and survival time show a very similar response pattern. A correlation coefficient between the two parameters is 0.98 and the "t" value of 9.23 ($n = 5$) indicates that the two are correlated with 99% confidence. Rate of mortality (1/ET50) and the rate of calcium decline are also correlated with 99% confidence ($n = 5$) and the correlation coefficient of 0.98 indicates that 98% of the variance in survival time with temperature is accounted for by the rate of decline of calcium concentrations in the plasma.

Although many biochemical, physiological, and histological disturbances occur as a result of cadmium poisoning in fish (Gardner and Yevich 1970; Larsson et al. 1976) one of these disturbances may ultimately be limiting and cause death. The extent of hypocalcemia observed in this experiment is extremely severe (40% reduction) and hypocalcemia in humans to a level of 3.5 meq/L (30% reduction) causes spontaneous discharge of motor and sensory nerves with symptoms of tetany and convulsions (Wintrobe et al. 1974, p. 1912). In view of the consistency of the extent of hypocalcemia with the symptoms of acute cadmium poisoning and the correlation between the rate of calcium decline and the rate of mortality, we believe that cadmium causes disturbances in ion regulation resulting in hypocalcemia which is severe enough to cause the death of the rainbow trout.

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Bioeconomic Model of the Gulf of Carpentaria Prawn Fishery

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CLARK, C. W., AND G. P. KIRKWOOD. 1979. Bioeconomic model of the Gulf of Carpentaria prawn fishery. J. Fish. Res. Board Can. 36: 1304–1312.

We present a bioeconomic model, of an intermediate degree of complexity and realism, of the prawn fishery of the Gulf of Carpentaria and adjacent waters in Northern Australia. The model employs 21 parameters to describe the performance of two classes of vessels exploiting several stocks of prawns. The model predicts the number of vessels of each class entering the fishery under free access, and the prediction is compared with available data. The model is also used to obtain estimates for the economically optimal number of vessels of each type.

Key words: model, economics of fisheries, limited entry, prawn fishery, Australia

CLARK, C. W., AND G. P. KIRKWOOD. 1979. Bioeconomic model of the Gulf of Carpentaria prawn fishery. J. Fish. Res. Board Can. 36: 1304–1312.

Nous présentons un modèle bioéconomique, plus ou moins complexe et réaliste, de la pêcherie de crevettes du golfe de Carpentaria et des eaux adjacentes de l'Australie septentrionale. Le modèle utilise 21 paramètres pour décrire la performance de deux classes de bateaux exploitant plusieurs stocks de crevettes. Il prédit le nombre de bateaux de chaque classe entrant dans la pêcherie sous un régime d'accès libre, prédiction que nous comparons aux données disponibles. Avec ce modèle, nous estimons le nombre optimal, au point de vue économique, de bateaux de chaque type.

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THE economic theory of commercial fisheries, initiated by Gordon (1954), predicts that under conditions of free access, entry to any particular fishery will expand to a level at which all economic rents become dissipated. In an optimally regulated fishery, on the other hand, entry would be restricted to the level at which economic rents would be maximized. Limited entry programs have recently been established in a number of commercial fisheries in Canada and elsewhere, but the problem of estimating the optimal number (and type) of vessels to be admitted to a given fishery has received limited attention. Such estimates must clearly be based upon a model of the fishery — a model incorporating the main biological and economic features of the fishery — in short, a bioeconomic model.

The purpose of this study is to present, as a case study, such a bioeconomic model pertaining to a recently developed prawn fishery in Northern Australia. In formulating this model we were fortunate in having available estimates of both biological and economic parameters for the fishery, although the study also revealed certain limitations and gaps in the available data. Because of these limitations we were forced to adopt a number of simplifying assumptions in formulating our model. Consequently the numerical estimates pertaining to the "optimal" number and deployment of vessels must be treated with reservations. But modeling is always an exercise in successive approximation; we hope that the present study will indicate how the modeller's art can be used to address the problem of improving the economic performance of commercial fisheries.

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The Gulf of Carpentaria Prawn Fishery

The fishery for prawns in the Gulf of Carpentaria (Fig. 1) began in the mid-1960s, following the discovery by a government research survey of fishable stocks of banana prawns (*Penaeus merguiensis*) in the eastern parts of the Gulf. The banana prawn fishery in the Gulf quickly developed into Australia's most productive prawn fishery. In addition, important but subsidiary fisheries for tiger prawns (*P. esculentus* and *P. semisulcatus*) and endeavor prawns (*Metapenaeus endeavouri* and *M. ensis*) were established in the Gulf of Carpentaria and adjacent waters to the northwest of the Gulf. Banana prawns are also taken in waters to the northwest of the Gulf.

In 1965 fewer than 10 trawlers exploited the Gulf banana prawn stocks, but by 1971 some 165 trawlers were participating in this fishery. The subsequent development of the fishery is shown in Table 1.

Two classes of vessels, brine trawlers and freezer trawlers (popularly known as "wet boats" and "dry boats," respectively), exploit the prawn stocks. Brine trawlers, so

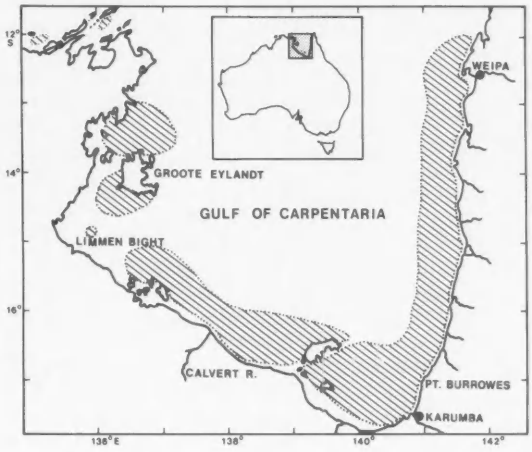

FIG. 1. Major prawn fishing grounds indicated by shaded area.

called because of their method of preserving the catch in tanks of cooled brine, are of relatively small size (<20 m). These vessels are primarily transient in the Gulf, travelling from home ports in Eastern Queensland to participate in the banana prawn season (March through July), with most then returning to their home ports and local fisheries. Because of the unsophisticated technique used for preserving prawns, and because of their limited capacity, the range of these vessels is restricted to within a few days' steaming (100–200 km) of the prawn unloading and processing facilities. The principal shore based facilities for banana prawns are at Karumba, although in the past there has been a facility at Weipa, and currently some brine trawlers unload to freezer trawlers.

TABLE 1. The Northern Australian prawn fishery, 1971–76: total number of vessels unloading prawns caught in the Gulf of Carpentaria between March and May (the peak of the banana prawn season) each year.

	Number of vessels		Catch (tonnes)		
	Brine trawlers	Freezer trawlers[a]	Gulf banana prawns	Other prawns[b]	Total prawns
1971	125	40	6500	3480	9980
1972	120	34	4990	2570	7560
1973	126	40	4570	3270	7840
1974	117	48	9710	4290	14000
1975	49	56	2200	2580	4780
1976	87	58	3680	2590	6270

[a]Between 1971 and 1974 some foreign built and crewed trawlers are included. These have been phased out of the fishery and replaced by Australian built and crewed trawlers.

[b]Other catches include the total catch of tiger and endeavor prawns north of 20°S, and catches of banana prawns outside the Gulf of Carpentaria.

Freezer trawlers, on the other hand, do not face such restrictions; their greater size (20–30 m) and their much greater endurance and capacity enable them to remain on the fishing grounds for months at a time, snap freezing their catch as it is taken. They also produce a superior and more highly valued product to that of the brine trawlers due to the immediate snap freezing. After participating in the Gulf banana prawn fishery, these vessels generally remain in northern waters for the remainder of the year, exploiting the alternative stocks of tiger and endeavor prawns (at a reduced rate of catch). As Table 1 indicates, the banana prawn fishery has experienced a progressive increase in the number of freezer trawlers, matched only partially by a decline in the brine trawler fleet. Because the capital costs of freezer trawlers are from 3 to 4 times those of brine trawlers, it can be seen that the level of capitalization in the fishery has increased since 1971. In March 1977, some 256 vessels were granted interim 3-yr licenses to fish for prawns in a declared fishing zone encompassing the Gulf of Carpentaria and waters to the northwest of the Gulf. (A few additional vessels were under consideration). The authorities have clearly stated that renewal of these licenses will not necessarily occur.

The banana prawns of the eastern Gulf of Carpentaria constitute by far the most important commercial prawn stocks in the north. Because of their somewhat unusual habit of forming dense schools, or "boils," these prawns provide exceptionally high catch rates. As a consequence, the banana prawn stocks are much more heavily exploited than are the stocks of alternative species. Fishermen consider access to the banana prawns to be essential to their economic well-being. Unfortunately, the stocks of banana prawns are subject to wide, unpredictable annual variation, as reflected in the annual catches shown in Table 1. A good banana prawn season produces high returns to vessel owners and crew (and also to the processing sector), but a poor season may result in net losses. Variability in recruitment to the banana prawn stocks has been a cause of continuing concern to the fishery.

Most of the prawns captured in northern Australia are exported in frozen form, Japan being the principal market. Export prices have remained generally high throughout the decade; correspondingly high ex-vessel prices (currently up to A $3.50 per kg. for brine-vessel banana prawns, and A $7.60 per kg for snap-frozen tiger prawns) prevail.

The general nature of our modeling problem can be inferred from these brief remarks. (See Copes 1977; and Lucas, Kirkwood, and Somers, unpublished data, for more detailed descriptions of the Northern Australian prawn fisheries.) The main problems we address are (a) the optimal allocation of effort, over time, between the various stocks of prawns, and (b) the long-run optimal number of vessels of each type. The solution of these problems will provide a basis for estimating the optimal number of vessel licences over the long run.

The Bioeconomic Model

The following equation has been employed as a model of the Gulf of Carpenteria banana prawn fishery:

$$(1) \quad \frac{dx}{dt} = -(M + F(t))x \quad (0 \le t \le T)$$

where

$x(t)$ = number of banana prawns in the stock at time t

TABLE 2. Parameter values employed in the model.

Biological parameters[a]	Technological parameters[a]	Economic parameters[b]
$M = 0.05$ wk^{-1}	$q_f = 1.79 \times 10^{-3}$ (vessel wk)$^{-1}$	$p_1{}^b = 0.90$ \$/kg
$k = 0.08$ wk^{-1}	$q_b = 1.38 \times 10^{-3}$ (vessel wk)$^{-1}$	$p_1{}^f = 1.10$ \$/kg
$t_o = -17.3$ wk	$s_f = 0.85$	$p_2{}^b = 1.10$ \$/kg
$w_\infty = 0.05$ kg	$s_b = 0.50$	$p_2{}^f = 1.50$ \$/kg
$\bar{R} = 3.7 \times 10^8$	$y_f = 1750$ kg (vessel wk)$^{-1}$	$c_1{}^f = 1600$ \$/vessel wk
	$y_b = 875$ kg (vessel wk)$^{-1}$	$c_2{}^f = 1600$ \$/vessel wk
		$c_1{}^b = 700$ \$/vessel wk
		$c_2{}^b = 700$ \$/vessel wk
		$c_F{}^f = 101\,000$ \$/vessel yr[c]
		$c_F{}^b = 10\,000$ \$/vessel yr

[a]Derived from Lucas, Kirkwood, and Somers (unpublished data).
[b]Derived from various economic surveys of the Northern Australian prawn fisheries; includes data to 1976 only. All figures are expressed in Australian dollars.
[c]Includes 10% annual interest on capitalized value of vessel.

t = time in weeks, with $t = 0$ corresponding to March 1
M = natural mortality rate (per week)
$F(t)$ = fishing mortality at time t (per week)
T = 1 yr, i.e. $T = 52$ wk.

Recruitment to the stock on March 1 is denoted by R:

$$x(0) = R. \tag{2}$$

Growth of individual prawns is modeled by the von Bertalanffy curve

$$w(t) = w_\infty\{1 - \exp(-k(t - t_o))\}^3 \tag{3}$$

where w_∞, k, t_o are parameters and $w(t)$ = average weight (kg) per prawn, at time t. Numerical estimates are available from biological investigations for each of the parameters M, w_∞, t_o, k, and $\bar{R}$ = average recruitment (Table 2).

Note that the model assumes a lack of coupling between banana prawn stocks in successive years. This concurs with the lack of any statistically significant correlation between escapement and subsequent recruitment for this population. If such a relationship were to exist, our model would require appropriate modification. The model also neglects any survival of adult banana prawns from one year to the next. Although there is in fact a small percentage survival (approximately 5% when unfished), neglecting it seems acceptable, at least as an approximation.

Fishing mortality $F(t)$ is assumed to be given by

$$F(t) = q_f E_1{}^f(t) + q_b E_1{}^b(t) \tag{4}$$

where q_f, q_b are catchability coefficients for freezer trawlers and brine vessels respectively, and where $E_1{}^f$, $E_1{}^b$ denote the effort levels in the banana prawn fishery for the two classes of vessels, at time t. Each vessel class can also exploit an alternative stock of prawns; as a simplifying assumption we suppose that these alternative stocks are distinct, viz. tiger and endeavor prawns, plus some additional banana prawn stocks, in northern Australia for freezer trawlers, vs. the prawn stocks of eastern Queensland for brine boats. Letting $E_2{}^f(t)$, $E_2{}^b(t)$ denote the levels of effort allocated at time t to the alternative fisheries, we then have constraints of the form:

$$E_i{}^f(t) \geq 0,\ E_i{}^b(t) \geq 0 \quad (i = 1, 2) \tag{5}$$

$$E_1{}^f(t) + E_2{}^f(t) \leq E_{\max}{}^f \tag{6}$$

$$E_1{}^b(t) + E_2{}^b(t) \leq E_{\max}{}^b \tag{7}$$

where $E_{\max}{}^f$, $E_{\max}{}^b$, represent total fleet capacity for the two vessel categories. We will assume that

$$E_{\max}{}^f = s_f Z^f \text{ and } E_{\max}{}^b = s_b Z^b$$

where Z^f, Z^b represent the actual numbers of freezer and brine vessels in existence, and s_f, s_b denote serviceability factors ($0 \leq s_f,\ s_b \leq 1$) representing the average fraction of time that vessels are actually available for fishing. Because of travel time to and from port, time for repairs, etc., these factors are significantly less than one.

We treat the fleet sizes Z^f, Z^b as parameters of the model, one of our main objectives being to provide a method of determining the optimal fleet size for each class of vessel.

Ideally the alternative prawn stocks should also be described by appropriate biological models. Bearing in mind the limitations on available data, however, we shall henceforth suppress the biological dynamics of these alternative stocks. Rather, we shall suppose that each alternative stock complex can be exploited (within practicable effort levels) at a constant average catch per unit effort. Inasmuch as the Northern stocks of tiger and endeavor prawns are at present only lightly exploited, this assumption appears quite realistic for these stocks. It may be considerably less realistic for the Queensland stocks, but we possess no data on this aspect.

The fixed-pool assumption also has the advantage of greatly simplifying our mathematical optimization prob-

lem. Indeed, without such an assumption the optimization problem becomes analytically formidable.

Neglecting fixed costs for the moment, we can express net annual economic revenue accruing from the combined prawn fisheries in the following form:

$$(8)\quad V\{E_1^f, E_2^f; E_1^b, E_2^b\} = \int_0^T \{[p_1^f q_f E_1^f(t) + p_1^b q_b E_1^b(t)]x(t)w(t) - c_1^f E_1^f(t) - c_1^b E_1^b(t) + (p_2^f y_2^f - c_2^f)E_2^f(t) + (p_2^b y_2^b - c_2^b)E_2^b(t)\}dt$$

where

p_1^f, p_1^b = landed price of banana prawns ($/kg)
p_2^f, p_2^b = landed price of alternative stocks ($/kg)
y_2^f, y_2^b = average catch rate per unit effort of alternative stocks (kg/vessel wk)
c_i^f, c_i^b = operating costs of effort ($/vessel wk).

Estimated values for these parameters (using data up to 1976) are given in Table 2.

We shall consider the following optimization problems. First, assuming given fleet sizes Z^f, Z^b, we determine the optimal allocation, over time, of effort between the various prawn stocks. In particular, we determine the optimal opening date for the banana prawn season, as a function of the model parameters and fleet sizes. Next we determine the long-run optimal fleet sizes for both types of vessel. The model also yields a prediction of the sizes of the fleets under conditions of unrestricted entry, and this prediction provides an independent test of validity of the model.

Before proceeding to the solution of these optimization problems, we comment further on the assumptions underlying the model. First, the banana prawn population has been represented (Eq. 1) by a single aggregated variable, x. In reality, the population is spread over a rather wide geographical area, a fact which influences the relative efficiency of the two types of vessels, here assumed to be a fixed constant. Brine boats, for example, are less costly than freezer trawlers, but can only operate effectively in close proximity to processing plants or other unloading points possessing freezing facilities. Secondly, annual recruitment to the banana prawn stocks has been represented (Eq. 2) as a constant R, whereas in fact recruitment is highly variable and unpredictable. Finally, total fishing mortality F has been represented (Eq. 4) as a linear function of the nominal effort levels E_1^f, E_1^b of the two vessel classes. In practice it seems likely that this relationship would involve some degree of nonlinearity resulting from saturation and crowding of vessels. Unfortunately the present data base for the banana prawn fishery provides little hope of estimating parameters for a model including spatial effects, stochastic recruitment, or nonlinearities. The consequences of these necessary simplifications will be discussed later.

The Case of a Single Fleet

For simplicity of exposition, we first consider the case in which only brine boats (say) participate in the fishery. Our optimization problem then becomes:

$$(9)\quad \underset{E_1, E_2}{\text{maximize}} \int_0^T \{(p_1 qxw - c_1)E_1 + (p_2 y_2 - c_2)E_2\}dt$$

subject to

$$(10)\quad \dot{x} = -Mx - qE_1 x, \quad x(0) = R$$

$$(11)\quad 0 \le E_1(t), E_2(t); E_1(t) + E_2(t) \le E_{\max}.$$

This problem is readily solved on the basis of the maximum principle (Pontrjagin et al 1962). However, since the results will have a clear intuitive explanation, the reader who is not interested in the mathematical details can brush over the following derivation.

The appropriate Hamiltonian is:

$$(12)\quad H(x, E_1, E_2, \lambda) = (p_1 qxw - c_1)E_1 + (p_2 y_2 - c_2)E_2 + \lambda(-Mx - qE_1 x)$$

$$(13)\quad = \sigma_1(t)E_1 + \sigma_2 E_2 - \lambda Mx$$

where λ denotes the adjoint variable, and the coefficients $\sigma_1(t)$, σ_2 are given by

$$(14)\quad \sigma_1(t) = p_1 qw(t)x(t) - q\lambda(t)x(t) - c_1$$

$$(15)\quad \sigma_2 = p_2 y_2 - c_2.$$

In these expressions, the adjoint variable $\lambda = \lambda(t)$ represents the "shadow price" of the banana prawn resource at time t. (The absence of a shadow price for the alternative stock is a consequence of our assumption that the fishery fails to deplete this stock significantly.)

The Pontrjagin maximum principle specifies the following necessary conditions for an optimal solution:

$$(16)\quad \frac{d\lambda}{dt} = -\frac{\partial H}{\partial x} = -(p_1 qw - q\lambda)E_1 + M\lambda$$

$$(17)\quad \lambda(T) = 0$$

(18) E_1 and E_2 must maximize $H(x(t), E_1, E_2, \lambda(t))$ over the constraint set of Eq. (11) for each t

From these conditions it is possible, as we now show, to obtain a complete description of the optimal effort allocation policy.

The appropriate technique for solving linear optimization problems of this kind is first to determine any "singular" solutions, which arise if either of the functions $\sigma_1(t)$ or σ_2 vanishes over a time interval of positive length. Now σ_2 is a constant, and clearly the case $\sigma_2 \le 0$ can be discarded because in this case no positive revenue can ever be obtained by exploiting the alternative pool: in this case $E_2 \equiv 0$, and the existence of the alternative pool is irrelevant. Henceforth therefore we may assume that

$$(19)\quad \sigma_2 > 0.$$

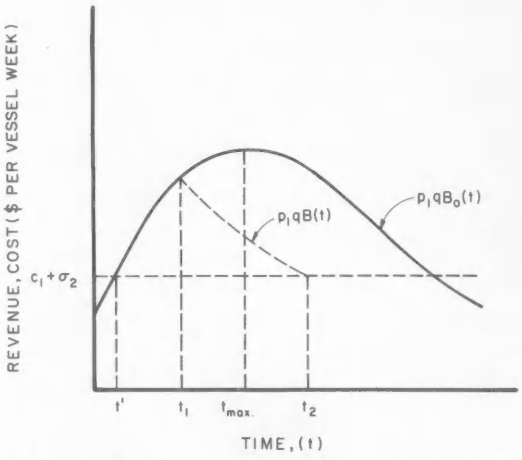

FIG. 2. Optimal fishing (single fleet case). The curve labelled $p_1qB_0(t)$ corresponds to unfished biomass; $p_1qB(t)$ corresponds to optimal fishing. The fishing season extends from t_1 to t_2. The level $c_1 + \sigma_2$ represents opportunity cost; fishing is only profitable when p_1qB lies above this level.

From Eqs. (10), (14), and (16) we obtain, after some simplification,

$$\dot{\sigma}_1 = p_1qx(t)(\dot{w}(t) - Mw(t)). \quad (20)$$

Thus $\dot{\sigma}_1$ can only vanish at $t = t_{\max}$, where $\dot{w}(t_{\max}) = Mw(t_{\max})$. (Note that $t_{\max}$ is the date of maximum biomass $x(t)w(t)$ of the banana prawn stock, Fig. 2.) Hence our problem has no singular solution.

Condition (18) implies that, at each specific time t, the linear form $\sigma_1(t)E_1 + \sigma_2E_2$ must be maximized by choice of an effort allocation satisfying $E_1, E_2 > 0$, $E_1 + E_2 \leq E_{\max}$. This trivial linear-programming problem has the solution:

$$(21) \quad \begin{cases} E_1(t) = 0 \text{ and } E_2(t) = E_{\max} \text{ if } \sigma_1(t) < \sigma_2 \\ E_1(t) = E_{\max} \text{ and } E_2(t) = 0 \text{ if } \sigma_1(t) > \sigma_2 \end{cases}$$

Thus at any time t, *the entire fleet capacity must be allocated to one or the other of the two stocks*; it is never optimal to allocate a part of the fleet to both stocks at the same time. However, as noted further in the discussion at the end of the paper, this conclusion may be subject to question, at least for the case of brine vessels, because of various aspects of the fishery that have been suppressed as a result of our simplification.

Next we determine the optimal times t_i at which the fleet should switch from one stock of prawns to the other. It follows readily from Eq. (20) that there can exist at most two such switches, at (say) times t_1 and t_2. Let us assume (as is in fact the case) that at the end of the calendar year ($T = 52$) the fishery for banana prawns is less profitable than that for the alternative stocks, i.e.

$$p_1qx(T)w(T) - c_1 < p_2y_2 - c_2. \quad (22)$$

Now since $\lambda(T) = 0$, Eq. (22) implies that $\sigma_1(T) < \sigma_2$. By continuity we have, for some $t_2 < T$:

$$\sigma_1(t) < \sigma_2 \text{ for } t_2 \leq t \leq T \text{ and } \sigma_1(t_2) = \sigma_2 \quad (23)$$

(unless the banana prawn stocks never become profitable). By Eq. (21) the fishery switches from banana prawns ($E = E_{\max}$) to the alternative stocks ($E_2 = E_{\max}$) at time t_2.

Since $E_1(t) = 0$ for $t_2 < t \leq T$, Eqs. (16) and (17) now imply that $\lambda(t) \equiv 0$ for $t_2 \leq t \leq T$. By (14) we have therefore

$$\sigma_1(t_2) = p_1qw(t_2)x(t_2) - c_1 = \sigma_2. \quad (24)$$

Thus the optimal switch occurs precisely at the instant t_2 when the banana prawn stocks have been depleted to a level of equal profitability with the alternative stocks (Fig. 2). This solution is also clear on intuitive grounds: the banana prawn season terminates as soon as the returns fall below those that can be achieved from the alternative stocks, or in other words, as soon as the gross rate of return to effort (p_1qxw) falls below the opportunity cost of harvesting ($c_1 + \sigma_2$).

An important consequence of this result is that (under the assumptions of our model) the switchover from the banana prawns to the alternative stocks does not require government regulation, but will automatically take place in an optimal manner. (This may no longer be valid if recruitment of banana prawns becomes affected by the fishery, or if the alternative pool becomes significantly depleted.)

On the other hand, the opening date t_1 for the banana prawn fishery clearly does require regulation. This can be seen formally as follows: First it is intuitively clear that the shadow price $\lambda(t)$ is positive for all $t < t_2$ (this is fairly easy to prove from the differential equation (16)). Now a well known characteristic of common-property resource exploitation is its inevitable neglect of the shadow price of the resource stock. Thus the unregulated fishery operates as if $\lambda(t) \equiv 0$, i.e. it sets (suboptimally)

$$\sigma_1(t) = p_1qw(t)x(t) - c_1.$$

Thus, according to the switching law (21), the unregulated fishery switches from the alternative stocks to the banana prawn stocks at the instant t' when

$$p_1qw(t')x(t') - c_1 = \sigma_2,$$

or in other words, as soon as banana prawns become more profitable than the alternative stocks. But since $\lambda(t') > 0$, the optimal switching time occurs at some later date t_1. This optimal opening date t_1 can readily be determined by numerical simulation of the model:

Number of vessels	Opening date (calculated on a weekly basis)
0–90	March 1
100–140	March 8
150–200	March 15

We note that t_1 depends upon the fleet capacity Z; as one would expect, the larger the fleet capacity, the shorter the season.

The solution to our optimal effort allocation problem can be described readily in terms of Fig. 2. In this Figure the solid curve labeled $p_1qB_o(t)$ represents the *natural biomass curve* (multiplied by the constant factor p_1q) where $B_o(t)$ is given by the equation

$$B_o(t) = Re^{-Mt}w(t)$$

Thus $B_o(t)$ represents the biomass of the unexploited banana prawn stock. This curve reaches a peak at $t_{\max} = 4.3$, i.e. on April 2. The dashed curve $p_1qB(t) = p_1qx(t)w(t)$ represents the banana prawn biomass resulting from an optimal fishing policy. The full effort capacity $E_{\max}$ is devoted to the banana prawn stock during the season from t_1 to t_2; this season brackets the biomass peak date $t_{\max}$.

To determine the optimal fleet size (brine vessels only), let $V(Z)$ denote the maximum value of the objective, Eq. (9), where $E_{\max} = sZ$. With c_F denoting total annual fixed costs per vessel (capital costs, insurance, etc.) net revenue for the fishery equals

$$V(Z) - c_FZ$$

The optimal fleet size $Z = Z^*$ is then given by

$$(25) \quad \frac{dV}{dZ} = c_F$$

The solution of this equation is readily obtained in conjunction with the simulation program used to determine the optimal dates t_1 and t_2.

The Case of Two Fleets

We proceed now to the general problem of allocation of the two fleets. Similar calculations to those given above immediately show that (a) at any time t, each fleet should be completely allocated to the banana prawn stocks or to its particular alternative, and (b) each fleet should (and can be expected automatically) to switch from banana prawns to its alternative at the moment when revenue flow declines to the opportunity cost level for that fleet. Hence the optimal allocation problem reduces to the determination of optimal opening dates t_1^f, t_1^b for the banana prawn season for each vessel class.

In general these optimal opening dates need not be the same. Realistically, however, we cannot imagine that the operators of either vessel class would stand for an earlier opening date for vessels of the other calss. (In any case, the model is not highly sensitive to the opening date (March 1 or later).) Hence we shall only consider the (theoretically second best) case of a single opening date t_1, as before. Once again a simple simulation-and-search routine can be employed to optimize t_1 for any combination of Z^f and Z^b. This leads to a maximum value $V(Z^f, Z^b)$ for total annual revenue (neglecting fixed costs) for the entire fleet. Net annual revenue is then calculated by subtracting fixed costs:

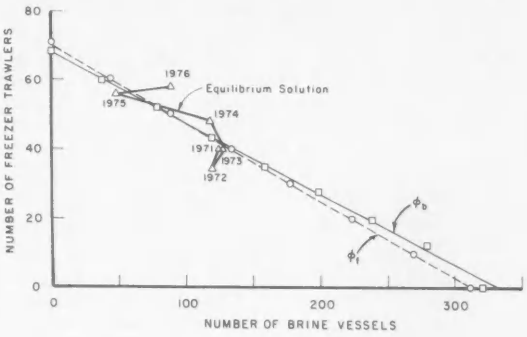

FIG. 3. Reaction curves and historical data. The curves ϕ_b, ϕ_f are the reaction curves for brine vessels and freezer trawlers, respectively. Their intersection is the predicted equilibrium of the open-access fishery (with opening date regulated to maximize economic yield.

$$(26) \quad V_{\text{net}}(Z^f, Z^b) = V(Z^f, Z^b) - c_F{}^fZ^f - c_F{}^bZ^b.$$

Numerical Results

Equation (26) can be employed for two purposes, (a) prediction of the fleet sizes Z^f, Z^b that will result in equilibrium from unrestricted entry of vessels, and (b) estimation of the economically optimal fleet sizes, i.e. those that maximize net annual revenue. For the predictive result we first derive the *reaction curve* for each vessel type (Fig. 3). For example, the curve ϕ_b (Fig. 3) represents the number of brine vessels Z^b entering the fishery, assuming a given number Z^f of freezer trawlers. According to Gordon (1954), this number will be characterized by the fact that net economic revenue for the brine vessel fleet will equal zero, i.e. the brine vessel fleet will be in *bionomic equilibrium*. Thus $\phi_b(Z^f)$ is equal to the fleet size Z^b such that $V_b(Z^f, Z^b) - c_F{}^bZ^b = 0$, where $V_b(Z^f, Z^b)$ denotes the total annual revenue accruing to the brine vessel fleet. The reaction curve ϕ_f is defined similarly.

These reaction curves turn out to be particularly sensitive to the values of the fixed-cost parameters $c_F{}^f$, $c_F{}^b$. Unfortunately, these parameters are difficult to specify with any precision, since both depend on nonmarket decisions taken by vessel owners. For brine vessels, we assume that fixed cost consists of the actual cost of steaming from eastern Queensland and return (estimated at approx. $2000), plus an unknown "relocation opportunity cost" which represents the minimum expected profit that will induce brine vessel owners to undertake the trip to the Gulf. An appropriate value for this relocation cost was then determined indirectly, by tuning the model so that the reaction curve ϕ_b provided a good fit for the data of Table 1 (see Fig. 3). This gave the value $c_F{}^b = \$10{,}000$, i.e. a re-

TABLE 3. Net annual economic revenue, $V_{net}(Z^f, Z^b)$ as a function of the number of freezer trawlers, Z^f, and of brine boats, Z^b, in millions of dollars (Australian).

Number of brine boats (Z^b)	Number of freezer trawlers (Z^f)									
	0	10	20	30	40	50	60	70	80	90
0	—	1.66	2.15	2.09	1.74	1.23	0.62	−0.05	−0.76	−1.50
20	0.25	1.66	2.02	1.89	1.50	0.96	0.34	−0.40	−1.05	−1.78
40	0.46	1.63	1.87	1.67	1.25	0.67	0.06	−0.62	−1.32	−2.05
60	0.62	1.58	1.71	1.46	0.99	0.41	−0.21	−0.90	−1.60	−2.32
80	0.74	1.51	1.54	1.23	0.74	0.16	−0.48	−1.15	−1.85	−2.56
100	0.82	1.42	1.36	1.00	0.47	−0.11	−0.75	−1.43	−2.11	−2.82
120	0.86	1.31	1.17	0.77	0.22	−0.35	−0.99	−1.66	−2.37	−3.08
140	0.88	1.18	0.97	0.53	−0.01	−0.61	−1.25	−1.92	−2.60	−3.33
160	0.86	1.04	0.77	0.28	−0.23	−0.83	−1.47	−2.15	−2.85	−3.54
180	0.82	0.88	0.55	0.08	−0.48	−1.09	−1.72	−2.37	−3.10	−3.78
200	0.76	0.72	0.33	−0.14	−0.68	−1.29	−1.97	−2.62	−3.29	−4.03
300	0.12	−0.20	−0.66	−1.18	−1.77	−2.43	−3.04	−3.76	−4.40	−5.14

location opportunity cost of approximately \$8000. Note that $c_F{}^b$ is not assumed to include interest (or depreciation) on vessel capital, since in most cases the brine vessels were built to operate in the Queensland fishery, and enter the Gulf fishery in an opportunistic fashion.

For freezer trawlers, fixed cost $c_F{}^f$ consists of "basic cost" (annual repairs, maintenance, depreciation, insurance) plus interest on invested capital. It was found that a 10% annual rate of interest led to a reasonable fit for the reaction curve ϕ_f (see Fig. 3); this figure may seem low, but it may realistically reflect the fact that processing companies are willing to accept minimal returns on their own trawler operations and rely on processing to produce a profit. The resulting value is $c_F{}^f$ = \$101,000, of which \$90,000 represents "basic cost."

Net annual revenue $V_{net}(Z^f, Z^b)$ as calculated from Eq. (26) is shown in Table 3. These results indicate an optimal prawn fleet consisting of 24 freezer trawlers and no brine trawlers, with net annual revenue equal to $V_{net}(24,0)$ = \$2.17 million, or \$86,000 per vessel. By contrast, a revenue maximizing fleet consisting solely of brine vessels would contain 140 vessels and earn a net revenue of \$0.88 million, or \$5,600 per vessel. Note, however, the importance of our assumption of a relocation cost of \$8,000 for brine vessels. If this cost is not subtracted from gross earnings, the 140 vessels would earn a net revenue of \$2.11 million; the maximum revenue would be \$2.5 million, involving 350 brine vessels. If relocation cost is totally disregarded, our model thus implies an optimal fleet consisting of 350 brine vessels and no freezer vessels.

It is clear that our model identifies no definitive advantage in terms of economic efficiency for either class of vessel; brine vessels have an advantage if the dislocation and discomfort of fishermen are disregarded; otherwise freezer trawlers seem to have an advantage. As noted elsewhere, freezer trawlers posess various other advantages, not all of which are encompassed by our model.

The model's prediction for equilibrium fleet composition under unlimited access is given by the intersection of the reaction curves ϕ_b, ϕ_f (see Fig. 3):

$$Z^b = 90 \quad Z^f = 50.$$

(Slight shifts in ϕ_b or ϕ_f would move this point considerably, but the new position would be somewhere in the vicinity of the curves shown in Fig. 3.) Our model thus appears to support the theory of common-property fisheries, in showing that unlimited access results in a significant excess of fishing capacity.

However, the claim that the optimal fleet would consist of just 24 freezer trawlers and no brine vessels cannot be taken very seriously, for various reasons. First, as already noted, the apparent relative inefficiency of brine vessels depends strongly on our assumed relocation opportunity cost of \$8000. If this figure is reduced to \$3500, brine vessels and freezer trawlers become roughly equivalent in efficiency, with one freezer trawler being equivalent to 10 brine vessels. Moreover, brine vessels do possess unmodeled relative advantages operating in proximity to processing plants. Secondly, the known variability of annual recruitment probably implies that a somewhat larger fleet is desirable; furthermore, our estimate of $\bar{R}$ (which affects the optimum) may be inaccurate, as we note below. Finally, our model entirely ignores the economic benefits accruing to the processing sector from the supply of prawns delivered by the trawlers. If these benefits were included in the analysis, the optimal fleet size would be increased. Unfortunately, we have not been able to estimate these benefits; according to the sensitivity results reported below, if benefits to the processing sector can be represented as a percentage (Δ) of the dockside value of the catch, then the corresponding increase in optimal fleet size would be given approximately by (1.7) × Δ (see Table 5).

Sensitivity Analysis

From the viewpoint of the fishery, one of the most

TABLE 4. Sensitivity of model predictions with respect to average recruitment of banana prawns.

Model prediction	Elasticity[a] with respect to recruitment $\bar{R}$
Optimal fleet size (freezer trawlers)	1.3
Net economic revenue	2.5
Optimal catch of banana prawns	1.0
Brine-vessel bionomic equilibrium	2.1
Freezer trawler bionomic equilibrium	1.7

[a]The elasticity of $f(x, y, \ldots)$ with respect to x, defined as $e_x = f^{-1}\partial f/\partial x$, represents the percentage increase in f resulting from a 1% increase in x.

TABLE 5. Sensitivity of optimal fleet size (freezer trawlers) to various parameters of the model.

Model parameter(s)	Elasticity of optimal fleet size
Price of prawns (all stocks)	+1.7
Operating costs	−0.6
Fixed costs	−1.1
Recruitment	+1.3
Natural mortality rate	−0.6

economically important factors is the annual level of recruitment (R) to the banana prawn stocks. Our average value $\bar{R} = 3.7 \times 10^8$ is based on a very short time series of preliminary estimates, namely

$$R_{1974} = 7.1 \times 10^8, R_{1975} = 1.5 \times 10^8, R_{1976} = 2.6 \times 10^8.$$

These have standard deviation

$$S_R = 3.0 \times 10^8.$$

Although valid estimates for R are not available for earlier years, the catch data of Table 1 suggest that long-run average recruitment may be somewhat greater than the assumed $\bar{R}$. The sensitivity of our model with respect to $\bar{R}$ was therefore tested, with the results shown in Table 4. These results show that, for example a 10% error in the value of $\bar{R}$ results in approximately a 13% error in the estimated optimum fleet size, and approximately a 20% error in the predictions of fleet size under unregulated bionomic equilibrium. This emphasizes the significance of recruitment to the fishery.

The sensitivity of the model to various other parameters is indicated in Table 5. We note that the sensitivity to prawn prices is high (+1.7), but is negated by sensitivity to total costs (−1.7). Thus, although current (1979) price and cost levels are considerably greater than those quoted in Table 2, the overall effect on our analysis should be relatively small.

Discussion

One consequence of the model's simplicity is that, for any given set of parameter values, the computed optimum fleet always consists exclusively of vessels of one type or the other. Which class is preferred depends particularly on the value of $c_F{}^b$, the brine-vessel fixed-cost parameter, which includes a "relocation opportunity cost" term. If this relocation cost exceeds $3500 per vessel per annum, the optimum fleet consists entirely of freezer vessels, and conversely.

Recent developments in the fishery seem to indicate that neither of the vessel types described by our model is optimal, but that a less expensive, somewhat smaller freezer trawler is more appropriate. Thus many brine vessels are currently being fitted with freezing facilities, while newly constructed freezer trawlers are of smaller dimension than formerly. However, it is probably advisable to retain some vessels of both types in the fishery, since each vessel type possesses certain advantages — brine vessels are less costly whereas freezer trawlers are most flexible, for example.

A second simplification concerns linearity of the model, which gives rise to the conclusion that optimal allocation requires the entire fleet to be allocated to one or the other of the socks at any one time. In practice this situation may be suboptimal for various reasons. First, crowding and interference of vessels on the banana prawn grounds may result in diseconomies. Secondly, a high catch rate by the brine vessel fleet may result in saturation of unloading facilities. And finally, since individual vessels compete for the catch of banana prawns, there may be an incentive for vessel owners to employ economically excessive levels of inputs to ensure as large a share of the catch as possible. These effects might be overcome in principle by means of a scheme of allocated quotas allowing each vessel a limited share of the banana prawn catch. However, in view of the unpredictability of recruitment of banana prawns, we feel that such measures (which might be strongly resisted by fishermen) might be more costly than beneficial.

Finally, our estimate of the optimal long-term fleet size is based on an assumption of constant annual recruitment R, which as we have noted is quite unrealistic. Treating R as a random variable would require an estimate of its probability distribution, but this is not likely to become available for several years. Since the present fleet size is apparently well above the long-term optimum, this problem is not an urgent one.

Future research to alleviate some of the shortcomings of the present model would include: additional biological study pertaining especially to recruitment, in order to obtain an improved estimate of $\bar{R}$; more detailed, comprehensive, and updated economic surveys, including in particular the processing sector of the fishery.

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Overdominance of a Muscle Protein (*Mp*-1) Locus in the Japanese Oyster, *Crassostrea gigas* (Ostreidae)

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BUROKER, N. E. 1979. Overdominance of a muscle protein (*Mp*-1) locus in the Japanese oyster, *Crassostrea gigas* (Ostreidae). J. Fish. Res. Board Can. 36: 1313–1318.

A microgeographical population genetic study of the Japanese oyster, *Crassostrea gigas*, revealed a case of overdominance for a muscle protein (*Mp*-1) locus. This locus consists of a two allele polymorphism. A study of the relative "fitness" of the three genotypes (Mp-$1^{105/105}$, Mp-$1^{105/100}$, and Mp-$1^{100/100}$) revealed a heterozygote superiority in the segment of the oyster population at the lowest tidal level (+ 1.2 m). In segments of the population at higher tidal heights (i.e. + 1.8 and + 2.4 m), there appears to be a selective advantage for Mp-1^{105} allele since the highest "fitness" values belong to the Mp-$1^{105/105}$ genotype. Since oysters do not appear at the field station lower than 1.2 tidal m, it could not be determined if the Mp-1^{100} allele had a selective advantage at tidal levels lower than 1.2 m. However, the Mp-1^{105} allele possibly exhibits a selective advantage in the subtropical marine environment of southern Japan, while the Mp-1^{100} allele is favored in the temperate marine environment of northern Japan. This suggests perhaps that a micro- and macrogeographical cline in gene frequency exists for the *Mp*-1 locus due to a thermal gradient.

Key words: Japanese oyster, *Crassostrea gigas*, population genetic study, overdominance, heterozygote superiority, *Mp*-1 locus, micro- and macrogeographical cline, thermal gradient, tidal height, latitude

BUROKER, N. E. 1979. Overdominance of a muscle protein (*Mp*-1) locus in the Japanese oyster, *Crassostrea gigas* (Ostreidae). J. Fish. Res. Board Can. 36: 1313–1318.

Lors d'une étude de la génétique d'une population microgéographique d'huîtres japonaises, *Crassostea gigas*, on a découvert un cas de surdominance d'un locus de protéine musculaire (*Mp*-1). Ce locus consiste en un polymorphisme de deux allèles. Une étude de la « bonne forme » relative des trois génotypes (Mp-$1^{105/105}$, Mp-$1^{105/100}$ et Mp-$1^{100/100}$) indique une supériorité de l'hétérozygote dans le segment de la population d'huîtres au niveau de marée le plus bas (+ 1,2 m). Dans les segments de la population aux hauteurs de marée supérieures (i.e. + 1,8 et + 2,4 m), il semble y avoir avantage sélectif pour l'allèle Mp-1^{105}, étant donné que les valeurs maximales de « bonne forme » appartiennent au génotype Mp-$1^{105/105}$. Comme les huîtres sont absentes de la station située au-dessous de 1,2 m de marée, nous n'avons pu nous assurer si l'allèle Mp-1^{100} avait un avantage sélectif à des niveaux de marée inférieurs à 1,2 m. Cependant, l'allèle Mp-1^{105} peut avoir un avantage sélectif dans l'environnement marin subtropical du Japon méridional, alors que l'allèle Mp-1^{100} est favorisé dans l'environnement marin tempéré du Japon septentrional. Ceci donne à croire qu'il existe peut-être un cline micro- et macrogéographique de fréquence des gènes pour le locus *Mp*-1, résultant d'un gradient thermique.

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THE Japanese oyster, *Crassostrea gigas*, is indigenous to the Japan Archipeliago, Korea, China, and Asiatic Russia (Stenzel 1971). Since the early 1900s, this species has been shipped annually from Japan to the West Coast of North America for use in a commercial fishery. In both Canada and the United States the species reproduces naturally and has become well established in British Columbia and Washington State (Cahn 1950; Quayle 1969; Wesley 1975).

The marine environment along the eastern coastline of northern Japan (i.e. the Miyagi Prefecture) is remarkably different from that of southern Japan (i.e. the Hiroshima Prefecture). Warm water from the Kuroshio current dominates the southeastern coastline while the cold Kurile current influences the north (cf. Ahmed 1975). Consequently, the eastern coastline of Japan belongs to two different zoogeographical regions which are inhabited by distinctly different species groups (Ekman 1953).

A genetic similarity (Nei 1972) of 0.988 was found between a northern temperate population of *C. gigas* at Miyagi (38.3°N; surface water temperature ~10°C in February and 20°C in August) and a southern sub-

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tropical population at Hiroshima (34.2°N; surface water temperature ~15°C in February and 25°C in August) based on the comparison of 23 protein synthesizing loci (Buroker et al. 1979a). However, there exists a two allele muscle protein polymorphism at the *Mp*-1 locus which displays a substantial allele frequency difference between the two populations (Buroker et al. 1979a). Since this was the only polymorphic locus out of the eleven studied which displayed a significant difference between the populations, selection could be responsible for favoring different alleles in the two zoogeographical regions of Japan.

Evidence for selection in natural populations may consist of: (1) macrogeographical clines (e.g. spatial changes of allele frequencies with concomitant geographical variation; Koehn 1969; Johnson 1971; Schopf and Gooch 1971; Powell 1971); (2) microgeographical clines (e.g. changes in allele frequencies with concomitant microgeographical gradients or with local environmental heterogeneity; Hamrick and Allard 1972; Levinton 1973; Koehn et al. 1973; Boyer 1974); (3) generation clines (i.e. a progressive change in allele frequencies with year-class or increasing age; Fujino and Kang 1968; Koehn et al. 1971; Tinkle and Selander 1973; Koehn et al. 1976). It is felt that a macrogeographical cline could explain the *Mp*-1 allele frequencies differences for the Japanese population of *C. gigas*. However, further evidence in the form of (2) and (3) would certainly verify this assertion. Here I examined the genic variation at the *Mp*-1 locus and provide evidence for a microgeographical and generation cline in allele frequency at the *Mp*-1 locus.

Materials and Methods

An experimental Japanese oyster seed planting was made in 1969 at the Big Beef Creek, Hood Canal field station of the College of Fisheries, University of Washington. Since the initial planting, two major oyster sets (1971 and 1973) have occurred here. In Fig. 1, the three year-classes (1969, 1971 and 1973) have been partitioned according to shell length and intertidal location. A population genetic study was conducted on the basis of this partitioning.

In the summer of 1975, samples of *Crassostrea gigas* from the Big Beef Creek field station were collected from three distinct areas of the littoral zone (Fig. 1) representing the low extreme of the population, the middle of the population, and the high extreme of the population. At each of the three intertidal locations the oysters could be partitioned according to the 1969, 1971, and 1973 year-classes. This represents oysters of approximately 2, 4, and 6 years of age. Consequently, this partitioning of oyster samples resulted in three different age-groups from each intertidal location, or nine distinct subsamples collected at the field station.

Protein electrophoresis, using the methods of Buroker (1975, 1978) and Buroker et al. 1975, 1979a, b) was employed to examine the level of genetic variation of eleven polymorphic loci from these oysters. The 11 protein synthesizing loci (*Aat*-2, *Adk*-1, *Est*-2, *Lap*-1, *Lap*-2, *Mdh*-2, *Mp*-1, 6*Pgdh*, *Pgi*, *Pgm*-1, and *Sdh*) that were investigated, code for the enzymes adenylate kinase, asparate aminotransferase, esterase, leucine aminopeptidase, malate dehydrogenase, muscle protein, 6-phosphogluconate dehydrogenase, phosphoglucose isomerase, phosphoglucomutase, and sorbitol dehydrogenase.

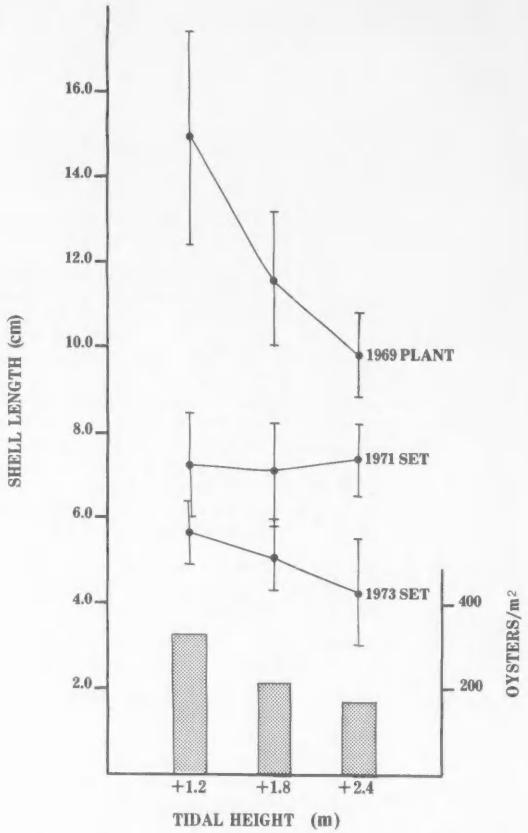

FIG. 1. The relationship between tidal height and oyster shell length recorded for three different year-classes (1969, 1971 and 1973) at the Big Beef Creek field station with the relative oyster density at each tidal level.

Results

A comparison of allelic frequencies for all but the *Mp*-1 locus revealed no significant difference between subsamples at the field station (i.e. no difference between age-groups or tidal height). However, the *Mp*-1 locus revealed very apparent differences in allelic frequencies between subsamples (Table 1). With increasing tidal height and increasing oyster size (age), there appears to be a selective advantage for the M_r·1^{105} allele. Based on genotype frequencies calculated from the observed genotypes in Table 1, relative survival values were computed between year classes at each of three tidal heights. The survival values were computed

TABLE 1. The observed and Hardy–Weinberg expected genotypic values, allele frequencies and standard errors, chi-square values for goodness-to-fit of observed to expected genotypes, and statistical probability levels for an electrophoretic study of the muscle protein locus (*Mp*-1). of the Japanese oyster, at three different intertidal heights.

		Tidal height (metres)					
		+1.2		+1.8		+2.4	
Year-class	Genotype	Obser.	Expect.	Obser.	Expect.	Obser.	Expect.
1969 plant	105/105	9	9.521[a]	13	13.128	21	21.262
	105/100	34	32.958	38	37.744	41	40.475
	100/100	28	28.521	27	27.128	19	19.262
	Total	71	71.000	78	78.000	81	81.000
	Allele frequencies and standard errors:						
	105	0.366 ± .040		0.410 ± .039		0.512 ± .039	
	100	0.634 ± .040		0.590 ± .039		0.488 ± .039	
	Chi-square[a]	0.071		0.004		0.014	
	Prob. (df = 1):	$P > 0.70$		$P > 0.90$		$P > 0.90$	
1971 set	105/105	7	9.620	12	10.843	19	19.512
	105/100	39	33.760	36	38.313	42	40.976
	100/100	27	29.620	35	33.843	21	21.512
	Total	73	73.000	83	83.000	82	82.000
	Allele frequencies and standard errors:						
	105	0.363 ± .040		0.361 ± .037		0.488 ± .039	
	100	0.637 ± .040		0.639 ± .037		0.512 ± .039	
	Chi-square:	1.759		0.303		0.051	
	Prob. (df = 1):	$P > 0.10$		$P > 0.50$		$P > 0.80$	
1973 set	105/105	14	11.813	9	8.679	10	8.337
	105/100	35	39.375	36	36.643	31	34.327
	100/100	35	32.813	39	38.679	37	35.337
	Total	84	84.000	84	84.000	78	78.000
	Allele frequencies and standard errors:						
	105	0.375 ± .037		0.321 ± .036		0.327 ± .038	
	100	0.625 ± .037		0.679 ± .036		0.673 ± .038	
	Chi-square:	1.037		0.026		0.732	
	Prob. (df = 1):	$P > 0.30$		$P > 0.80$		$P > 0.30$	

[a]The expected genotypic values used in the chi-square tests are based on the gene frequencies computed from the observed genotypes of each year-class at the intertidal collecting localities.

between the original 1969 oyster plant and the natural 1971 and 1973 oyster sets, and between the natural 1971 and 1973 oyster sets. From the survival values the relative "fitness" of each genotype at the three tidal heights was computed (Table 2).

When relative "fitness" is compared to tidal height, a heterozygote superiority for the *Mp*-$1^{105/100}$ genotype is evident at the lowest tidal level (+1.2 m). Also, at this tidal height, it would appear that the "fitness" of the *Mp*-$1^{105/105}$ genotype is declining while that of the *Mp*-$1^{100/100}$ genotype is continuing to increase. It can be inferred from this data that the heterozygote superiority indicates overdominance for the *Mp*-1 locus of *C. gigas*. In classical cases of overdominance where two alleles are selectively maintained by heterozygote advantage, a stable equilibrium exists:

(1) $p = S_2/S_1 + S_2$ (Kimura and Ohta 1971),

where S_1 is the coefficient of selection against one homozygote (*Mp*-$1^{105/105}$), S_2 is the coefficient of selection against the other homozygote (*Mp*-$1^{100/100}$), and p is the frequency of the *Mp*-1^{105} allele. If such a stable equiuilibrium exists at the lowest intertidal level of the population, then the coefficients of selection computed from the three year-classes at the 1.2-m tidal level (Table 2) can be used to calculate the equilibrium gene frequency. When S_1 equals 0.201 and S_2 is 0.129, the equilibrium gene frequency of the *Mp*-1^{105} allele computes to 0.391. A visual examination of Table 1, indicates that the *Mp*-1^{105} allele frequency for each of the three year-classes at the 1.2-m tidal height fall very near the equilibrium gene frequency. Further, with increasing tidal height the *Mp*-1^{105} allele frequency deviates from the equilibrium gene frequency. Chi-square analysis verifies these observations (Table 3). In other words, some environmental selection parameter (e.g. salinity,

TABLE 2. Genotypic frequencies of three year classes (1969 plant, 1971 and 1973 natural oyster sets) obtained at different intertidal levels.

	1.2-m tidal height		
Genotype	105/105	105/100	100/100
Frequency of:			
1969 plant	0.127	0.479	0.394
1971 set	0.096	0.534	0.370
1973 set	0.167	0.417	0.417
Relative survival values:			
1969/1971	1.323	0.897	1.065
1969/1973	0.760	1.149	0.945
1971/1973	0.575	1.281	0.887
Cummulative value:	2.658	3.327	2.897
Relative fitness:	$\frac{2.658}{3.327}=0.799$	$\frac{3.327}{3.327}=1.000$	$\frac{2.897}{3.327}=0.871$
Selection coefficients:	0.201	0.000	0.129
	1.8-m tidal height		
Genotype	105/105	105/100	100/100
Frequency of:			
1969 plant	0.167	0.487	0.346
1971 set	0.145	0.434	0.422
1973 set	0.107	0.429	0.464
Relative survival values:			
1969/1971	1.152	1.122	0.820
1969/1973	1.561	1.135	0.746
1971/1973	1.355	1.012	0.909
Cummulative value:	4.068	3.269	2.475
Relative fitness:	$\frac{4.068}{4.068}=1.000$	$\frac{3.269}{4.068}=0.804$	$\frac{2.475}{2.475}=0.608$
	2.4-m tidal height		
Genotype	105/105	105/100	100/100
Frequency of:			
1969 plant	0.259	0.506	0.235
1971 set	0.232	0.512	0.256
1973 set	0.128	0.397	0.474
Relative survival values:			
1969/1971	1.116	0.988	0.918
1969/1973	2.023	1.275	0.496
1971/1973	1.813	1.290	0.540
Cummulative value:	4.952	3.553	1.954
Relative fitness:	$\frac{4.952}{4.952}=1.000$	$\frac{3.553}{4.952}=0.717$	$\frac{1.954}{4.952}=0.395$

TABLE 3. Chi-square analyses to test the hypothesis that the observed genotypes for different tidal heights and year-classes (Table 1) are the same as the Hardy–Weinberg expected values assuming an equilibrium allele (Mp-1^{105}) frequency of 0.391.

	Chi-square values, df = 1		
Tidal height (metres):	+1.2	+1.8	+2.4
Year-class:			
1969 plant	0.424	0.245	10.207[a]
1971 set	2.069	0.930	6.468[a]
1973 set	1.205	3.526	3.580

[a]Statistically significant ($P<0.05$).

water temperature, etc.) produces an equilibrium condition when the frequency of the Mp-1^{105} allele equals 0.391. At tidal heights above and below 1.2 m, the environmental conditions at the field station are such to cause a condition of disequilibrium which would result in the fixation of the Mp-1^{105} allele with increasing tidal height and in the fixation of the Mp-1^{100} allele with decreasing tidal height (Table 2). The equilibrium condition may in part be responsible for a greater abundance of oysters at the 1.2-m tidal height than at the other levels (cf. Fig. 1).

This condition of overdominance which maintains the polymorphism at the Mp-1 locus creates a segrega-

tional load (Crow 1958) on this oyster species. This load (L_{od}) equals

$$S_1S_2/S_1 + S_2$$

where S_1 and S_2 are as in equation 1. Consequently, the segregational load is 0.079, when S_1 equals 0.201 and S_2 is 0.129. This means that for every 100 zygotes produced per generation in a population of *C. gigas* approximately eight will die a genetic death due to the maintenance of this polymorphic locus.

Discussion

The discovery that a two allele polymorphism at the *Mp*-1 locus is maintained by overdominance (i.e. a form of balancing selection) provides new insight into why the two large geographical populations (Miyagi and Hiroshima) from Japan exhibit remarkably different allele frequencies for this gene. The equilibrium condition brought about by the environmental selective parameter which produces an equilibrium allele frequency (Mp-1^{105}) of 0.391 in the microgeographical study must also be present on a macrogeographical level in Japan to maintain a two allele polymorphism in both the Miyagi and Hiroshima populations. If this condition in Japan were not present then the *Mp*-1 locus should be fixed for one or the other of the two alleles in each geographical population. This is not the case. The equilibrium condition that would maintain the heterotic *Mp*-1 locus in Japan must exist somewhere between the northern Miyagi population and the southern Hiroshima population since their Mp-1^{105} allele frequencies are 0.307 and 0.704, respectively. Recalling that the thermal environmental conditions are very different between coastal waters of northeastern and southeastern Japan, it can be suggested that temperature is responsible for maintaining the *Mp*-1 locus in a heterotic state. Water temperature can also be used to explain the microgeographical cline of the Mp-1^{105} allele frequency in the littoral zone at the Big Beef Creek field station. During complete water cover in the summer months of the Pacific northwest an increase in water temperature is related to increasing tidal height (Quayle 1969). This thermal cline through the littoral zone exists because solar radiation creates high temperatures in the upper water layers which supply water for organisms of the high littoral zone. Consequently, oysters along the high littoral zone would experience warmer temperatures than those from the low littoral zone. Apparently, this warmer water condition results in a selective advantage for the Mp-1^{105} allele as reflected by the "fitness" of the Mp-$1^{105/105}$ homozygote.

The evidence as presented would suggest that a latitudinal thermal cline exist for the muscle protein locus of *C. gigas*, where the Mp-1^{105} allele is favored in warm water environments and the Mp-1^{100} allele is favored in cooler environments. Although further latitudinal population genetic studies are needed to verify this assumption, such macrogeographical clines in gene frequency due to thermal gradients have been documented for many aquatic fauna (Koehn and Rasmussen 1967; Koehn 1969; Schopf and Gooch 1971; Johnson 1971, 1974, 1977; Merritt 1972; Mitton and Koehn 1975) as well as at least one microgeographical case (Johnson 1971).

Although the *Mp*-1 locus displays a classical case of overdominance, it appears to be the exception and not the rule for other polymorphic loci in the Japanese oyster. It is the only case out of 11 polymorphic loci in the combined studies at the field station and in the Japanese populations which display a clear heterozygote superiority; or including monomorphic loci, one heterotic locus out of 23 investigated (Buroker et al. 1979a). Population genetic studies on 16 Ostreidae species from three genera (*Crassostrea*, *Ostrea*, and *Saccostrea*) (Buroker 1975, 1978; Buroker et al. 1975, 1979a, b), reveal that the *Mp*-1 polymorphism is unique to the Japanese oyster, *C. gigas*.

A mortality of approximately 8% for all new zygotes per generation represents a rather high cost of maintaining this two allele polymorphism. Although the average fecundity of *Crassostrea gigas* is about 56 million eggs per female (Galtsoff 1964), the Japanese oyster in an effort to maintain high levels of genetic variation (Buroker et al. 1975) could not afford the luxury of many such heterotic loci without soon becoming extinct.

Since the *Mp*-1 locus is detected by a general protein stain, very little can be concluded concerning the function and biochemical properties of the protein. However, it is the more anodal migrating protein and travels the greatest distance in a starch gel-matrix when compared to the other proteins. This suggests a rather low molecular weight for the protein. It appears to be tissue specific to adductor and heart muscle. Finally, the two banded heterozygote genotypes suggest that it is a monomeric protein (Buroker et al. 1975).

The implications of overdominance at the *Mp*-1 locus to the commercial Japanese oyster fishery are noteworthy. With the advent of the oyster hatchery, selective breeding can now be achieved to match the environmental conditions to the appropriate muscle protein genotype. For example, since the Mp-$1^{105/105}$ genotype has a selective advantage in the warm environment of the upper intertidal (Table 2), an oyster grower that plants seed and eventually farms this crop from the intertidal zone might place an order to a commercial oyster hatchery for seed having the favorable Mp-$1^{105/105}$ genotype for the warmer environment. On the other hand, the Mp-$1^{100/100}$ genotype is better suited to floating tray methods of culturing in which a cooler environment is created by continuous water cover and circulating water currents. Such a selective breeding program in Canada, Japan or the United States provides the opportunity for the commercial oyster grower to eliminate much of the 8% natural mortality created by the heterotic *Mp*-1 locus.

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Carcinonemertes errans and the Fouling and Mortality of Eggs of the Dungeness Crab, *Cancer magister*[1]

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WICKHAM, D. E. 1979. *Carcinonemertes errans* and the fouling and mortality of eggs of the Dungeness crab, *Cancer magister*. J. Fish. Res. Board Can. 36: 1319–1324.

Laboratory experiments analyzing the correlation between epibiotic fouling and mortality of the eggs of the Dungeness crab, *Cancer magister* Dana, reveal that both can result from predation on crab eggs by the nemertean *Carcinonemertes errans* Wickham. Fouling appears to be stimulated by the release of yolk material into the egg clutch through worm feeding and defecation. Significant correlations exist between worm density and both fouling and mortality in field samples. The ranking of the correlations between these three parameters suggest that nemertean activity is the causative agent and that fouling is only incidentally correlated to egg mortality through its correlation to worm density.

Key words: Carcinonemertes errans, *Cancer magister*, crab-egg fouling, crab-egg mortality

WICKHAM, D. E. 1979. *Carcinonemertes errans* and the fouling and mortality of eggs of the Dungeness crab, *Cancer magister*. J. Fish. Res. Board Can. 36: 1319–1324.

En analysant, dans des expériences de laboratoire, la corrélation entre les salissures épibiotiques et les mortalités d'oeufs de dormeur du Pacifique, *Cancer magister* Dana, on constate que les unes et les autres peuvent résulter de l'action prédatrice du némertien *Carcinonemertes errans* Wickham sur les oeufs du crabe. Ces salissures semblent être dues surtout à l'expulsion de vitellus parmi la masse d'oeufs à la suite de l'alimentation et de la défécation des vers. Il existe, dans des échantillons prélevés sur le terrain, des corrélations significatives entre densité des vers, d'une part, et salissures et mortalités, d'autre part. Les corrélations de rang établies entre ces trois paramètres donnent à croire que l'activité des némertiens est la cause principale des salissures, et que ces dernières ne sont liées à la mortalité des oeufs que fortuitement par le biais de sa corrélation avec la densité des vers.

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EPIBIOTIC fouling of the eggs of the Dungeness crab, *Cancer magister* Dana, was first described by Fisher and Wickham (1976). They found that eggs from crabs found outside the San Francisco Bay were often covered by dense growths of filamentous microorganisms and that egg clutches so fouled exhibited elevated levels of egg mortality. They suggested that the fouling was the cause of the egg mortality and that this pathological condition might be involved in the protracted decline suffered by the Central California crab fishery. Because crab eggs from the Eureka area to the north, which still had a productive fishery, were less fouled and displayed lower levels of mortality, they felt that the fouling might be the result of effluents from San Francisco Bay's major metropolitan system.

Fisher (1976) followed the initial field observations with a laboratory study which appeared to confirm the causal role of epibiotic fouling in the mortality of Dungeness crab eggs. Treatments which appeared to cause increases or decreases of fouling burdens, such as nutrient or antibiotic addition, also were correlated with increased or decreased average mortalities.

An important part of the argument linking fouling with mortality in Fisher's (1976) study was the fact that both fouling and mortality in his samples appeared to co-occur at the periphery of the egg clutch. This was similar to the nature of infestation of blue crab (*Callinectes sapidus*) eggs with the fungus *Lagenidium* (Rogers-Talbert 1948).

In all of the research on egg fouling mentioned above, including Rogers-Talbert's (1948) work, reference is made to the presence of worms of the genus *Carcinonemertes* in the crab egg clutches. *Carcinonemertes* has long been known as a symbiont on brachyuran crabs and Humes (1942) and Kuris (1971) suggested that it was a predator on the eggs of its hosts. I conducted a study redescribing the nemertean on the Dungeness crab as the new species, *Carcinonemertes errans*, specific to this host (Wickham 1978). During this study I was able to observe directly feeding on crab eggs by *C. errans* and confirm their role as an egg predator. The important part of these observations was that worms were seen feeding on clean, healthy eggs. Feeding began as soon as the worms migrated from the host exoskeleton to the host egg clutch after host oviposition.

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Carcinonemertes errans began to cause egg mortality before any fouling had occurred. Also a great deal of organic material was released into the egg clutch by defecation and puncture of crab eggs.

I undertook this study on feeding by *Carcinonemertes errans* on Dungeness crab eggs to determine whether the fouling and mortality of these eggs observed earlier might be caused by the worms, rather than by external sources such as pollutants from San Francisco Bay and whether predation by this worm contributed a significant amount of mortality to its host's eggs.

Materials and Methods

The relationship between worm activity and fouling was first investigated with isolated worms and crab eggs in petri dishes. Crab setae with approximately 50 eggs attached were placed in 50-mL petri dishes with seawater and exposed to 0, 1, 2, and 3 worms. Five replicates were held for 6 d and dead eggs were counted. Dead eggs were distinguishable as empty or partially emptied egg membranes. Fouling was quantified as in Fisher and Wickham (1976): A, no filaments observable at 100× magnification; B, occasional short filaments; C, majority of surface covered with short filaments; D, surface extensively covered with short and long filaments; E, surface completely covered with filaments and detrital material.

This experiment was repeated in stoppered test tubes which were darkened at the bottom 2 cm with externally applied black tape. *Carcinonemertes errans* is photonegative and darkening the bottom kept the worms in closer contact with the eggs than in the petri dishes. Setae with approximately 50 eggs each were exposed to 0, 1, and 2 *C. errans* in seven replicates for 10 d. Dead eggs were counted and fouling was quantified by removing 10 eggs equidistant along each setae and examining them with a phase microscope at 500× magnification. All filaments attached along the egg circumference longer than 2 μm were counted and the average number of filaments per egg was computed.

Field samples of egg clutches were obtained from fishermen who removed a cluster of 20 000–40 000 eggs from individual crabs which was preserved in vials with 10% buffered formalin. The samples were removed from the crab egg-clutch with forceps which were inserted deep enough to remove entire egg-setae. Samples were collected from the waters off Eureka in northern California during January 1977. These samples were analyzed with the aid of a dissecting microscope as follows: worms were separated and counted, worm egg-strings (which are laid among the crab eggs) were also counted. The distance of worms and their egg-strings from the distal egg on the crab seta they were found on was measured in millimetres. The length of time the crab had brooded the egg clutch was estimated using a description of the embryological development of the Dungeness crab described in Wickham (1979). Crab egg mortality was computed by counting all live and dead eggs from a subsample of 10 arbitrarily selected egg setae (approximately 2000 eggs). The subsample was dried and weighed and the weight per egg was computed. The rest of the sample was dried and weighed and the number of eggs estimated by dividing the total sample weight by the weight per egg. Worm density was expressed on a per 1000 crab egg basis. The original San Francisco samples of Fisher and Wickham (1977) were reexamined for the above data and supplemented with extra samples obtained later from the San Francisco area. Fouling was quantified in field samples as in the laboratory studies using three eggs removed from the tip, middle, and base of five egg setae and counting the filaments for the average number of filaments per egg.

The average percent egg mortality was computed for samples from San Francisco (1974–79), Eureka (1974–79), and Washington (1975, 1979) and projected forward to mortality at the end of the brooding period as in Wickham (1979).

Correlation coefficients were computed between worm abundance, fouling, and crab egg mortality. The measure of worm abundance used included both the number of worms and worm egg strings per sample. Wickham (1979) found that worms increased their feeding rate when they began to lay their own eggs and that the number of worms plus worm egg strings was more closely correlated to the number of dead crab eggs in samples than just the number of worms. The following correlations were computed: number of worms plus worm egg-strings per sample vs. number of dead crab eggs, number of fouling filaments per sample vs. number of dead crab eggs, number of worms plus worm egg-strings per sample vs. number of fouling filaments. All data were transformed to common logarithms.

Separate plots were prepared of the regression of the number of fouling filaments per crab egg on the number of worms and worm egg-strings per 1000 crab eggs in samples for both Eureka and San Francisco to compare the relationship between fouling and worm density in both areas. Again data were transformed to common logarithms.

Results

The petri dish experiments showed that both the number of dead eggs and the proportion of eggs in more advanced fouling categories increased with the number of worms present (Table 1). These results were confirmed in the more controlled test tube experiments. Again the average number (±SE) of filaments per egg and the number (±SE) of dead eggs per sample of 50 eggs increased with the number of worms.

No. *C. errans*	Filaments per egg	Dead eggs per sample
0	2.01±0.53	0.0
1	10.44±1.73	6.0±1.29
2	49.80±13.30	9.9±0.96

Correlation coefficients computed with field data between worm abundance vs. fouling ($r = 0.758$), worm

TABLE 1. Comparison of average number (± SE) of dead eggs per sample of 50 eggs and percent of eggs in fouling categories A–E for petri dishes with 0, 1, 2, and 3 *C. errans* added.

No. worms	Percent eggs in fouling category					No. dead eggs per sample
	A	B	C	D	E	
0	56	20	24			0.6±0.45
1	69	7	17	7		2.0±1.12
2	6	4	53	30	7	4.2±0.65
3			19	59	22	5.8±1.8

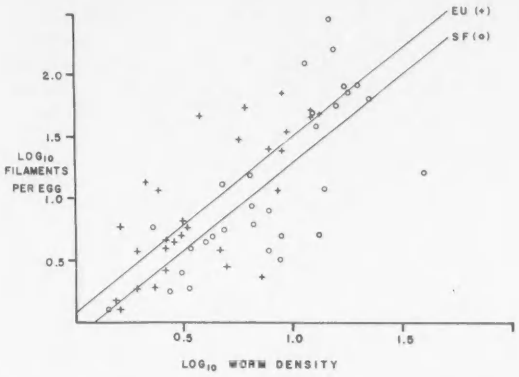

FIG. 1. Plot of the average number of filaments per egg against the number of worms plus worm egg strings per 1000 crab eggs in samples from Eureka and San Francisco. Data transformed to the common logarithm.

abundance vs. crab egg mortality ($r = 0.878$), and fouling vs. crab egg mortality ($r = 0.724$) were all significant ($P < 0.001$).

The regression of fouling on worm abundance in both Eureka and San Francisco appeared to be similar (Fig. 1). Ten samples were omitted from the San Francisco collection because of fouling loads too heavy to be accurately quantified. The average worm density for the omitted samples was 38.4 worms plus worm egg-strings per 1000 crab eggs.

The distribution of worms on the host egg setae differed between the San Francisco samples and the Eureka crabs (Fig. 2). Worms were found near the tips in San Francisco samples but were more evenly distributed in the Eureka samples. Worm egg strings were also more evenly distributed along the setae at both localities (Fig. 3).

The projected average host–egg mortality ranged from 10.6 to 63.3% with egg mortality increasing along with worm density (Table 2).

TABLE 2. Average worm density (worms per 1000 crab eggs) per host and average projected percent crab egg mortality from natural populations.

Sample collection		Worm density	Percent projected crab egg mortality	(*n*)
Eureka	1974–75	1.78	10.6	32
	1975–76	4.98	28.5	41
	1977–78	4.03	30.0	50
	1978–79	5.90	43.9	35
San Francisco	1974–75	14.59	63.3	37
	1975–76	8.56	50.3	86
	1976–77	10.02	45.1	44
	1977–78	24.96	62.5	48
Willapa Bay, Washington	1975–76	2.25	15.9	16
	1978–79	11.14	61.2	19

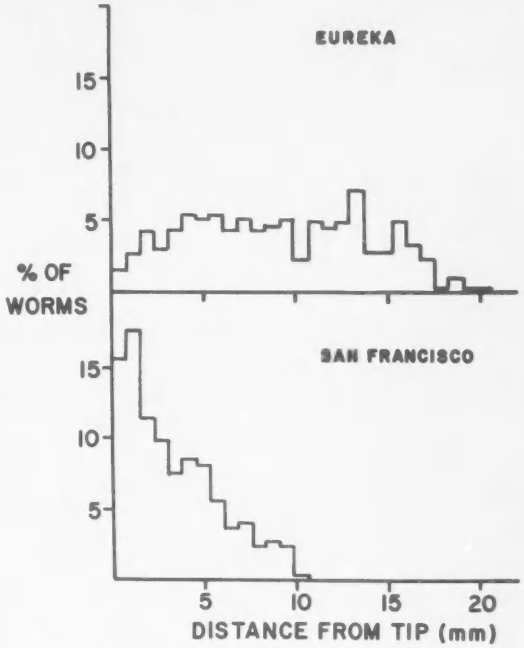

FIG. 2. Distribution of worms along the host egg-setae from San Francisco and Eureka samples.

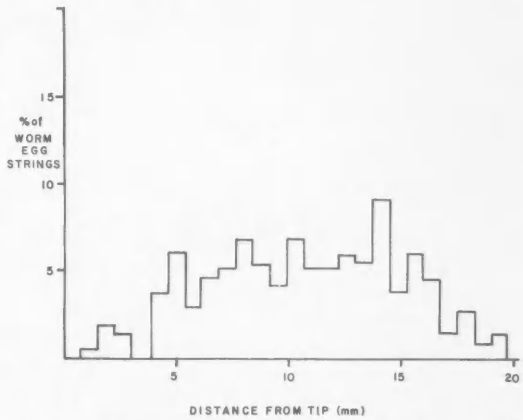

FIG. 3. Distribution of worm egg-strings along the host egg-setae from Eureka and San Francisco samples.

Discussion

The laboratory studies showed that *C. errans* was a direct source of filamentous fouling of crab eggs. Ob-

servations on the eggs during these experiments revealed that substantial quantities of worm feces were present. The level of nutrients would be directly proportional to the extent of worm feeding and fouling growth apparently responds to this. Mortality in these samples also showed that worms fed at a rate averaging 0.53 eggs per day.

A similar relationship between worm abundance and both fouling and mortality is apparent in the field samples. The fouling level was also correlated with crab egg mortality but the ranking of correlations showed that worm density has a significantly higher correlation to mortality than fouling does ($T_s = 2.54, P = 0.011$). Because fouling in the laboratory is caused by worm activity and is correlated in the field with worm density, it should also be secondarily correlated with egg mortality. However, the actual "cause" of egg mortality appears to be worm feeding.

Fisher (1976) concluded from his laboratory studies that fouling was a causative factor in Dungeness crab egg mortality. Unfortunately, no record was kept of worm abundance in his samples and because the experimental crabs were obtained from the Central California area, near the Russian River, worm density was likely to have been high. Crabs sampled at the same time showed a worm density of 4.13 *C. errans* per 1000 crab eggs (Wickham 1979). Because his samples lost a significant portion of their eggs clutches when they oviposited on flat aquaria substrata (I found that *C. magister* needs to oviposit on a sand substratum in order to form a burrow to hold the eggs until they have cemented to the setae), they probably had a clutch size of half that of normal crabs obtained in the field. This would mean that the effective density of worms on these crabs would be near 8.2 worms per 1000 crab eggs. Using the average mortalities reported by Fisher (1976) and an estimate of 8.2 worms per 1000 crab eggs gives a feeding rate of 0.47 eggs per worm per day, very close to the 0.53 found in the test tube study. Based on these calculations, almost all of the mortality observed in his study can be explained by worms.

Whether fouling, regardless of how caused, is actually capable of causing direct egg mortality is not clear. Fisher (1976) did find that egg mortality appeared to respond to manipulation of fouling. Again, interpretation is difficult without information on worm density. At least one treatment used, 1 mg/L malachite green, probably reduced mortality due to a deleterious effect on *C. errans*. I found in the laboratory that 1 mg/L malachite green caused these worms to contract immediately into a sphere and become inactive; 4 mg/L killed all worms within a few minutes.

The difference between the San Francisco and the Eureka crab populations is pertinent to the causal arguments put forth by Fisher (1976) and Fisher and Wickham (1976). They suggested the increased fouling outside San Francisco was due to unidentified factors in the effluent from San Francisco Bay. At the time of their early samples Eureka had both low fouling and low worm density. The samples obtained in 1977 from Eureka for this study showed increased worm density, apparently in response to a dramatic increase in host abundance (Wickham 1979). These Eureka samples showed that the regression of fouling on worm density was essentially identical in both localities. The Eureka samples were further along in the brooding period when sampled so the eggs would have been exposed to the worms for a longer time. This probably accounts for the slight elevation of the Eureka regression line over San Francisco. The 10 San Francisco samples omitted from the study due to their unquantifiably high fouling densities also had the highest worm densities so would probably not have affected the slopes of the regression lines, nor would they have altered their origins, which is more critical in this case.

If effluents from San Francisco had been involved in the fouling then, while the slopes of the regressions might have been similar, the origin of the regression line of fouling on worm density in San Francisco should have been greater than zero. Since that is not the case, it must be concluded that *C. errans* is the primary causative factor in the fouling observed in the field.

The differences observed by Fisher and Wickham (1977) in the mortality distribution in the egg clutches between San Francisco and Eureka are also probably best explained by differences in worm distributions. The distribution of worms in San Francisco samples was near the tips of egg setae, suggesting that mortality should also be greater there. The even distribution of mortalities through the egg clutch in the Eureka, Pacifica, and Russian River samples of Fisher and Wickham (1977) would not be expected if due to worms, except that the samples used from these regions were from more mature crab egg clutches and the worms had already begun laying their own eggs. The Eureka samples showed a more even distribution of worms along the egg setae. This distribution was similar to that of the worm egg-strings in both areas. Worms apparently begin to descend into the egg clutch as they mature. I have also observed a similar behavior in the species of nemertean which occurs on the crab *Cancer antennarius*. This worm secretes a mucous tube at the periphery of the host egg clutch in which it dwells for a portion of the host brooding period. Shortly prior to worm reproduction they were observed leaving their tubes and descending into the clutch where they then oviposited their own egg strings.

Only 35% of the worm populations in Fisher and Wickham's (1977) San Francisco (Drakes Bay) sample had begun reproducing while 96% of Eureka, 81% of Pacifica, and 57% of the Russian River samples had. The differences in the distribution of egg mortalities along the egg setae they had observed were, therefore, probably a sampling artifact resulting from the differences in maturity in *C. errans*. Also because young worms were near the tips of the egg setae, they probably were the source of the concentration of mortality at the tips observed by Fisher (1976) in the lab. His sampling

was done only for the first 3 wk of the brooding period so the distribution pattern of the worms present would have fit the mortality and fouling distribution he found.

The relationship between epibiotic fouling and mortality in infested hosts is well established in laboratory culture of larval crustaceans (Sindermann 1977). Mortality of fouled crustacean eggs, however, has been demonstrated only in instances of infection by fungal organisms capable of penetrating the egg membrane (Rogers-Talbert 1948; Anderson and Conroy 1968). Oppenheimer (1955) showed that pelagic fish eggs could be killed by epibiotic infestations, but this was probably due to interference with their flotation ability. Fisher's (1976) evidence for fouling-induced mortality in Dungeness crab eggs fails to account for the contribution of *Carcinonemertes* to the mortalities. A similar criticism could be weighed against Rogers-Talbert (1948). She implicated *Lagenidium* as the sole cause of egg mortality in blue crabs. In her paper, however, she included a plot of the percent of blue crabs infested with *C. carcinophila*. While she did not comment on it, the infestation rates of the fungus closely paralleled those of the worms, with the onset of fungal infestation following worm infestation by about 2 wk. The data she presented was insufficient to show any causal relation between nemerteans and fungus but probably warrants caution in interpreting her results.

While filamentous fouling of Dungeness crab eggs may be an extra source of mortality to those eggs, it appears to be more a result of direct predation on eggs by worms than a causative factor. The high levels of fouling and egg mortality found near San Francisco by Fisher and Wickham (1976) are probably due to epidemic levels of worms currently occurring there. Over the past 5 yr worm density has averaged approximately 14 per 1000 crab eggs resulting in an average crab egg mortality of greater than 55%.

Both Eureka and Washington Dungeness crab populations appear to have experienced an increase in worm density and host–egg mortality. Wickham (1979) suggested that these increases might be epidemic in nature resulting from the unusually high host densities which occurred in these regions during the sampling period. He suggested that the unstable oscillations in Dungeness crab abundance, possibly caused by the fishery (Botsford and Wickham 1978), led to these periods of abnormally high abundance (Fig. 4). Total worm abundance would increase during the years of peak crab abundance and then, when host abundance dropped, the worms would become concentrated on the remaining hosts, leading to very high densities of worms per host. The persistence of the high worm densities at San Francisco in the face of the reduced availability of hosts over the past 20 yr suggests that Dungeness crab and *Carcinonemertes errans* have reached a new equilibrium with worm predation now being the dominant biological control of crab abundance. Wickham (1979) showed that density effects on worm feeding and a patchy distribution on hosts prevented worms from consuming all their hosts' eggs and this inability to eradicate the host allowed the new equilibrium to persist.

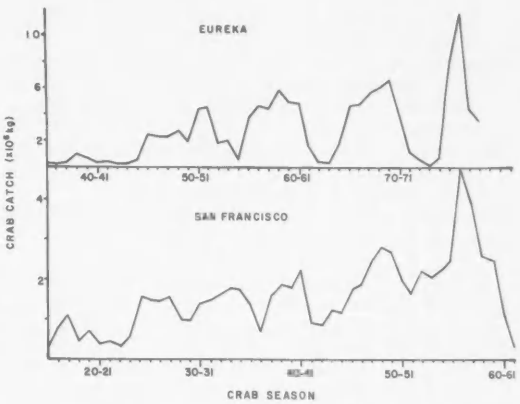

FIG. 4. Seasonal catches of Dungeness crab at San Francisco and Eureka, California, through the period of their most intense exploitation. The catch subsequent to 1961 at San Francisco has remained low averaging 250 000 kg.

If the high levels of fouling and crab egg mortality caused by *C. errans* at San Francisco are a new stable condition, and if Eureka and Washington are currently moving towards that condition, the entire Pacific coast Dungeness crab fishery could be in danger of an imminent collapse. The seriousness of the situation argues for a greatly increased effort on understanding the relationship between *Carcinonemertes errans* and its host.

Acknowledgments

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Le contrôle de la variabilité à court terme du phytoplancton estuarien: stabilité verticale et profondeur critique[1]

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FORTIER, L. ET L. LEGENDRE. 1979. Le contrôle de la variabilité à court terme du phytoplancton estuarien: stabilité verticale et profondeur critique. J. Fish. Res. Board Can. 36: 1325–1335.

Des séries temporelles de biomasse phytoplanctonique (ATP et chlorophylle *a*) ainsi que de capacité photosynthétique (assimilation du ^{14}C et production d'ATP en lumière saturante) ont été réalisées, au cours de l'été 1977, dans l'estuaire maritime du Saint-Laurent. La stabilité verticale de la colonne d'eau a été estimée par le nombre de Richardson. Les résultats permettent de conclure que le niveau moyen de la biomasse est une fonction inverse de la profondeur critique, dont les variations principales seraient liées au terme semi-mensuel du cycle de marée (M_f). Par ailleurs, la distribution fine de la biomasse et de l'activité photosynthétique est fonction de la stabilité de la colonne d'eau, qui varie selon le cycle semi-diurne de marée (M_2). Un modèle simple est élaboré à partir du modèle de la profondeur critique (Sverdrup), en associant les fluctuations en M_2 de la stabilité (importance du mélange vertical) aux variations en M_f de la profondeur critique. Les augmentations de biomasse, observées en eaux stables et claires, ne peuvent être expliquées par les taux de croissance calculés à partir de l'assimilation de ^{14}C mais correspondent par contre aux taux mesurés par la production d'ATP. L'analyse spectrale des séries de température et du nombre de Richardson suggère que la stabilité de la colonne d'eau est un facteur dont les variations temporelles de basse fréquence amplifient l'hétérogénéité spatiale d'un paramètre actif comme le phytoplancton, alors qu'un contaminant passif du milieu comme la température ne répond pas à de telles variations. Il s'ensuit que le spectre de la stabilité permet d'étendre aux basses fréquences l'étude du contrôle physique de la dynamique phytoplanctonique.

Mots clés: phytoplancton, estuaire du Saint-Laurent, variabilité, stabilité verticale, profondeur critique, nombre de Richardson, corrélation de Kendall avec retard, analyse spectrale

FORTIER, L. ET L. LEGENDRE. 1979. Le contrôle de la variabilité à court terme du phytoplancton estuarien: stabilité verticale et profondeur critique. J. Fish. Res. Board Can. 36: 1325–1335.

Hourly series of summer phytoplankton biomass (ATP and chlorophyll *a*) and photosynthetic capacity (^{14}C assimilation and ATP increase in saturating light) were sampled in the lower St. Lawrence Estuary during the summer of 1977. Vertical stability of the water column was estimated by the Richardson number. From the results it is concluded that the mean level of biomass is an inverse function of critical depth, likely linked to the neap-spring tidal cycle (M_f). On the other hand, the fine fluctuations of biomass and photosynthetic activity were related to vertical stability, which varies according to the semi-diurnal tidal cycle (M_2). A simple model was developed on the basis of Sverdrup's critical depth, combining the M_2 fluctuations in stability (degree of vertical mixing) to M_f variations in critical depth. Net biomass increases, observed in a nonturbid stabilized water column, cannot be explained by the growth rates computed from ^{14}C assimilation, but they are consistent with the measured rates of ATP production. Spectral analyses of time series of temperature and Richardson number suggest that low frequency water column stability variations amplify the spatial heterogeneity of a reacting parameter such as phytoplankton. On the other hand, a passive scalar such as temperature does not respond to these low frequency variations of stability. It follows that the study of stability spectra may give some insight into the low frequency control of phytoplankton dynamics.

Key words: phytoplankton, St. Lawrence Estuary, variability, vertical stability, critical depth, Richardson number, Kendall's cross correlation, spectral analysis

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LE concept de la profondeur critique, développé par Sverdrup (1953) à partir d'un modèle de Gran et Braarud (1935), fait ressortir l'importance de l'interaction entre le mélange vertical et la profondeur de la couche photique dans l'évolution saisonnière de la biomasse phytoplanctonique en milieu océanique. Dans leur étude sur la variabilité estivale du phytoplancton d'un milieu estuarien profond influencé par la marée, Winter et al. (1975) soulignent que « dans des conditions de stabilité modérée, lorsque la radiation solaire est suffisante et que la turbidité n'est pas excessive, le phytoplancton peut croître avec suffisamment de vigueur pour épuiser temporairement les sels nutritifs en surface ». Les résultats d'un travail similaire, réalisé par Sinclair (1978) dans l'estuaire maritime du Saint-Laurent, suggèrent que les fluctuations hebdomadaires de la biomasse et de la productivité phytoplanctoniques sont liées à la stratification verticale des eaux de surface.

Dans le golfe du Saint-Laurent, Platt (1972) démontrait, par l'analyse spectrale, que la variabilité spatiale de la concentration de chlorophylle est fonction d'une puissance de l'échelle spatiale. Ultérieurement, Denman et Platt (1975) suggéraient que la similtude observée à des échelles intermédiaires (100–1000 m), entre les spectres de chlorophylle et de température, pouvait être expliquée en termes du contrôle de la distribution de ces deux variables par les processus physiques. Cette similitude des deux spectres a été confirmée à diverses échelles pour différents milieux (Powell et al. 1975; Fasham et Pugh 1976; Lekan et Wilson 1978). A des échelles supérieures (> 1–2 km), la divergence observée entre le spectre de la chlorophylle et celui de la température a été attribuée aux processus de croissance du phytoplancton. Selon Platt (1975) et Denman et Platt (1975), l'hétérogénéité observée dans la distribution spatiale du phytoplancton, à ces échelles, est la résultante de l'équilibre entre les processus biologiques qui contribuent à la structuration spatiale et les processus turbulents qui tendent à disperser les structures formées. Cette hypothèse a été confirmée, pour un milieu côtier non estuarien, par les travaux récents de Therriault et al. (1978): leurs résultats indiquent que les phénomènes de turbulence, induits en surface par le vent, contrôlent effectivement les processus biologiques pendant les périodes de vent violent (> 4–5 $m \cdot s^{-1}$).

Dans un fluide stratifié et en mouvement, la mesure directe du mélange vertical doit tenir compte du degré de stratification du fluide et de l'importance de la turbulence générée à l'interface des couches se déplaçant à des vitesses différentes. Ce concept, développé en météorologie par Richardson (1920), a ensuite été introduit en océanographie estuarienne par Bowden (1963) ainsi que Bowden et Sharaf el Din (1966). Sa formulation physique, le nombre de Richardson, a été utilisée avec succès par Ganf (1974) pour expliquer les variations journalières de la distribution verticale du phytoplancton dans un lac équatorial peu profond.

En 1975, le GIROQ mettait sur pied un programme intensif d'échantillonnage (ECOVARIATE) afin d'étudier, dans l'estuaire du Saint-Laurent, la variabilité fine des paramètres physiques, biologiques et chimiques. Les premiers résultats de ce programme ont mis en évidence l'influence du régime des marées sur la distribution de la biomasse phytoplanctonique à court terme (Fortier et al. 1978) ou à moyen terme (Demers et al. 1979; Lafleur et al. 1979) et sur la capacité photosynthétique du phytoplancton (Demers et Legendre 1979). Le présent travail vise à caractériser la réponse du phytoplancton aux variations de la profondeur critique et du mélange vertical, induites à moyen terme par les phénomènes de marée, dans l'estuaire maritime du Saint-Laurent.

Matériel et méthodes

ECHANTILLONNAGE ET ANALYSES EN LABORATOIRE

Au cours de l'été 1977, deux séries temporelles de 116 et de 149 heures furent respectivement réalisées aux stations ECO-II et RA-PBI de l'estuaire maritime du Saint-Laurent. La station ECO-II (69° 19.5′W, 48° 10.5′N) fut occupée du 20 juillet à 20:00 (Heure normale de l'Est) au 25 juillet à 15:00 et RA-PBI (68° 31.0′W, 48° 34.5′N) du 16 août à 15:00 au 22 août à 19:00. Les deux stations se situent sur l'isobathe de 60 m, à la limite du plateau peu profond qui caractérise le côté sud de l'Estuaire et de la dépression du chenal laurentien plus au nord (fig. 1).

Des échantillons d'eau, pour la détermination en laboratoire de l'adénosine triphosphate (ATP), de la chlorophylle *a*, du carbone particulaire et de la production primaire à saturation lumineuse, furent prélevés à toutes les heures, à la profondeur de 2 m. Simultanément, des échantillons d'eau non filtrée, des profondeurs de 2, 5, 10, 20, 30 et 40 m étaient congelés pour l'analyse ultérieure des nitrates et des phosphates.

La température, la conductivité, la vitesse et la direction du courant furent enregistrées à intervalles de 1.25 min au moyen de courantomètres Aanderaa munis de sondes STP, immergés à 2, 5, 10, 16.5, 35 et 52.5 m à ECO-II et à 2, 5, 10 et 30 m à RA-PBI. A la suite d'incidents techniques, les valeurs de vitesse à 30 m (nécessaires au calcul du nombre de Richardson) et de production primaire à saturation ne sont pas disponibles pour la station RA-PBI.

Le dosage de l'ATP a été effectué selon la méthode décrite par Holm-Hansen et Booth (1966). Les deux duplicata (100 mL) étaient préfiltrés (soie #0, vide de maille: 569 μm) afin d'éviter la contamination par les plus gros individus du zooplancton. La technique fluorimétrique de Yentsch et Menzel (1963), telle que modifiée par Holm-Hansen et al. (1965), a été utilisée pour le dosage de la chlorophylle. Le carbone particulaire a été évalué par oxydation au dichromate (Strickland et Parsons 1972). La production d'ATP (Sheldon et Sutcliffe 1978) et l'assimilation du ^{14}C ont été choisies comme estimateurs de l'activité photosynthétique. La méthode décrite par Strickland et Parsons (1972) a été utilisée pour mesurer l'assimilation du ^{14}C, l'activité des filtres étant déterminée par scintillation liquide (Pugh 1973). Pour les deux méthodes (ATP et ^{14}C), des duplicata (125 mL) non préfiltrés étaient soumis à une incubation d'une durée de 4 h, dans des incubateurs de type Hawaian (Doty et Oguri 1959). A la première station (ECO-II), l'intensité lumineuse utilisée (33 $W \cdot m^{-2}$)

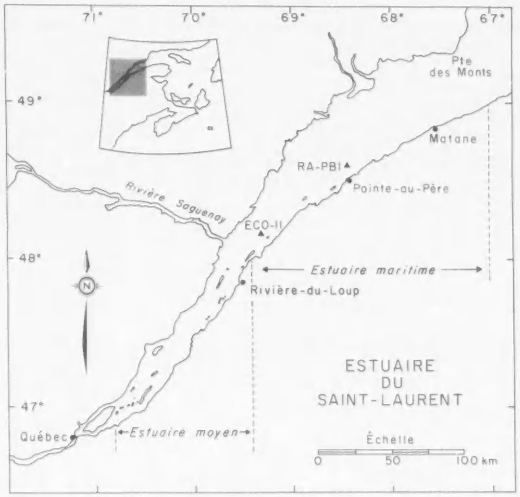

FIG. 1. L'estuaire du Saint-Laurent et la position des deux stations d'échantillonnage.

était suffisante pour assurer la saturation (Platt et Jassby 1976), alors qu'à la suite de difficultés techniques, elle était nettement insuffisante à la station RA-PBI ($7\ W \cdot m^{-2}$). Le refroidissement était assuré par circulation d'eau pompée à 2 m de profondeur. Les concentrations de nitrates et de phosphates ont été mesurées au moyen d'un autoanalyseur Technicon (Strickland et Parsons 1972).

A partir des mesures d'ATP, de chlorophylle a et de production primaire à saturation, deux indices physiologiques ont été calculés: (1) le rapport P/B, (ou "nombre d'assimilation") défini comme le taux d'assimilation du carbone à saturation lumineuse divisé par la chlorophylle a (mg C·mg chlorophylle $a^{-1} \cdot h^{-1}$); (2) le taux de croissance potentiel μ_{pot}:

$$\mu_{pot} = (1/t) \ln (1 + \Delta C/C)$$

où t représente le temps d'incubation, ΔC, l'augmentation de carbone et C, la biomasse initiale calculée en multipliant la concentration d'ATP par 250 (Hamilton et Holm-Hansen 1967). Ce taux de croissance potentiel a été estimé à partir des deux indices de productivité (ΔC) disponibles: la production d'ATP et l'assimilation du ^{14}C. Tel que formulé ici, μ_{pot} représente le taux exponentiel de croissance et s'exprime en h^{-1}.

Le nombre de Richardson (Ri) a été utilisé comme indice de la stabilité de la colonne d'eau:

$$Ri = \frac{g/\rho\ \delta\rho/\delta z}{(\delta v/\delta z)^2}$$ (Neumann et Pierson 1969)

où g représente l'accélération gravitationnelle, ρ, la densité de l'eau, $\delta\rho/\delta z$, le gradient vertical de densité et $\delta v/\delta z$, le gradient vertical de vitesse du courant. Tel que défini ici, Ri représente le rapport entre le travail de flottabilité, que tend à réduire le brassage vertical et dépend du degré de stratification de la colonne d'eau, et le travail de cisaillement (shear), qui tend au contraire à augmenter le brassage vertical et dépend du gradient vertical de vitesse. L'expression ci-dessus a été estimée, entre 2 et 35 m de profondeur, de la façon suivante:

$$\hat{Ri} = \frac{g/\bar{\rho}\ \Delta\rho/\Delta z}{(\Delta u/\Delta z)^2 + (\Delta v/\Delta z)^2}$$

où $\bar{\rho}$ représente la densité moyenne de la colonne d'eau, $\Delta\rho$, la différence de densité entre les deux profondeurs, Δz, la différence de profondeur, Δu, la différence entre les composantes Est (90°) de vitesse aux deux profondeurs et Δv, la différence entre les composantes Nord (0°) de vitesse. Cette formulation permet de tenir compte de l'orientation différente de l'écoulement des deux couches d'eau considérées.

Les profondeurs de 2 et 35 m ont été utilisées pour répondre à deux impératifs: (1) la différence de densité mesurée doit être suffisante pour atténuer l'impact des éventuelles erreurs de calibration des courantomètres et (2) l'écart entre les deux courantomètres doit être assez grand pour continuellement comprendre le niveau de la zone de cisaillement maximal, qui connaît des variations de profondeur importantes en fonction du cycle de marée.

TRAITEMENT STATISTIQUE

Les corrélations de Pearson (r) ont été utilisées lorsque les distributions des variables impliquées ne différaient pas significativement d'une loi normale ($P > 0.05$), selon le test de Kolmogorov–Smirnov. Dans le cas contraire, des corrélations non paramétriques de Kendall (τ) ont été calculées. L'étude de la structure des séries fut menée au moyen d'autocorrélations, dont les intervalles de confiance furent calculés selon la méthode décrite par Cox et Lewis (1966, p. 165). Lorsque nécessaire, des corrélations de Kendall avec retard ont été calculées entre les variables dont la distribution différait d'une loi normale. Ces corrélations de Kendall avec retard sont conçues sur le même principe que les corrélations avec retard habituelles, sauf que le τ de Kendall est calculé, à chaque pas, à la place du r de Pearson. Lorsque des variables physiques, enregistrées à toutes les 1.25 min, ont été comparées aux variables biologiques horaires, la moyenne des 13 valeurs adjacentes à l'heure du prélèvement biologique fut alors utilisée. Les valeurs horaires obtenues constituent donc une moyenne pour 15 min, ce qui permet d'atténuer la variabilité causée par les vagues à 2 m de profondeur.

Les spectres de variance du nombre de Richardson et de la température ont été calculés en utilisant la transformation rapide de Fourier (Cooley et Tukey 1965). Les séries de 8192 données (171 h) ont été subdivisées en 8 sous-séries de 1024 données (21 h). Le spectre lissé a été obtenu en faisant la moyenne des 8 spectres calculés sur les sous-séries.

Résultats

VARIABLES PHYSIQUES

Les variations temporelles de la profondeur des isothermes, isohalines et isopycnes (σ_t) sont illustrées à la fig. 2. Afin de faciliter l'interprétation des tracés, la variabilité attribuable au terme moyen lunaire (M_2) de la marée a été soustraite des données par moyennes mobiles de 12.5 h. A la station ECO-II, on observe, à partir de la 40ème heure environ, l'apparition en surface d'une masse d'eau de type continental caractérisée par des salinités faibles (< 29.5 ‰), des températures

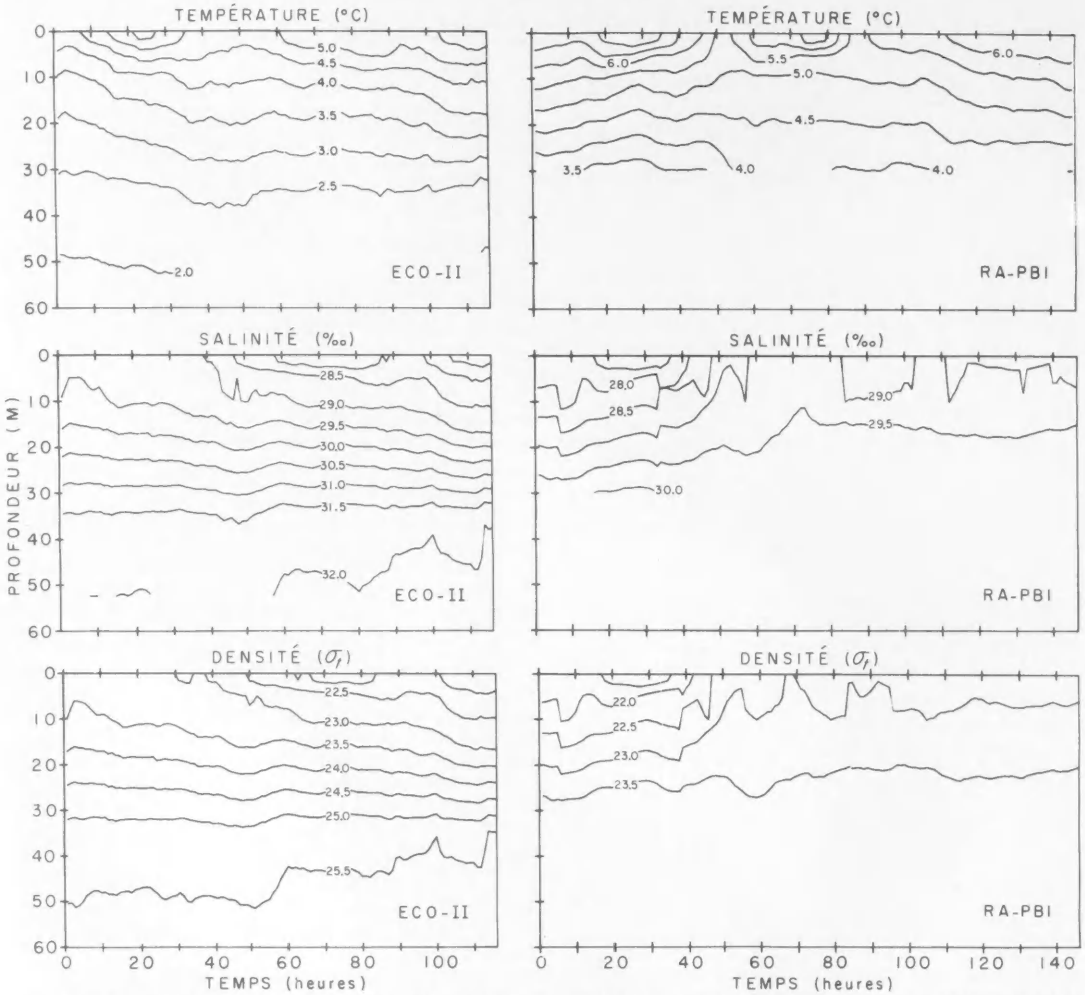

FIG. 2. Evolution horaire de la profondeur des isothermes, des isohalines et des isopycnes (σ_t), aux stations ECO-II (20–25 juillet 1977) et RA-PBI (16–22 août 1977) de l'estuaire maritime du Saint-Laurent. La composante semi-diurne M_2 a été extraite par moyennes mobiles.

relativement élevées pour l'Estuaire (> 4.5°C) et conséquemment des densités faibles ($\sigma_t < 23.5$). L'intrusion de cette masse d'eau continentale semble liée au terme semi-mensuel du cycle de marée (M_f), la série temporelle débutant en vive-eau et se terminant en morte-eau. A la station RA-PBI (fig. 2), le même type d'eau continentale se retrouve en surface ($t = 0$ à 54 h), mais cette fois associé à la période de vive-eau du cycle semi-mensuel de marée, c'est-à-dire, aux amplitudes quotidiennes maximales du niveau d'eau. A la présence en surface de ces lentilles d'eau continentale sont associées des augmentations importantes du gradient vertical de densité (fig. 3a, 3e).

Les variations temporelles du nombre de Richardson ($\hat{R}i$), à la station ECO-II, sont présentées à la fig. 4c. Il est à noter qu'une augmentation de $\hat{R}i$ correspond à une augmentation de la stabilité, alors que des valeurs faibles signifient un accroissement potentiel du mélange vertical. On remarque que, à partir de la 42ème heure, soit au moment où la masse d'eau continentale apparaît à la station, l'évolution du nombre de Richardson devient fortement structurée selon le cycle de marée et présente systématiquement une augmentation brusque au flot. Le corrélogramme de cette portion de la série révèle que la période du phénomène ($\simeq 12.5$ h) est égale à celle du terme moyen lunaire (M_2) du cycle de

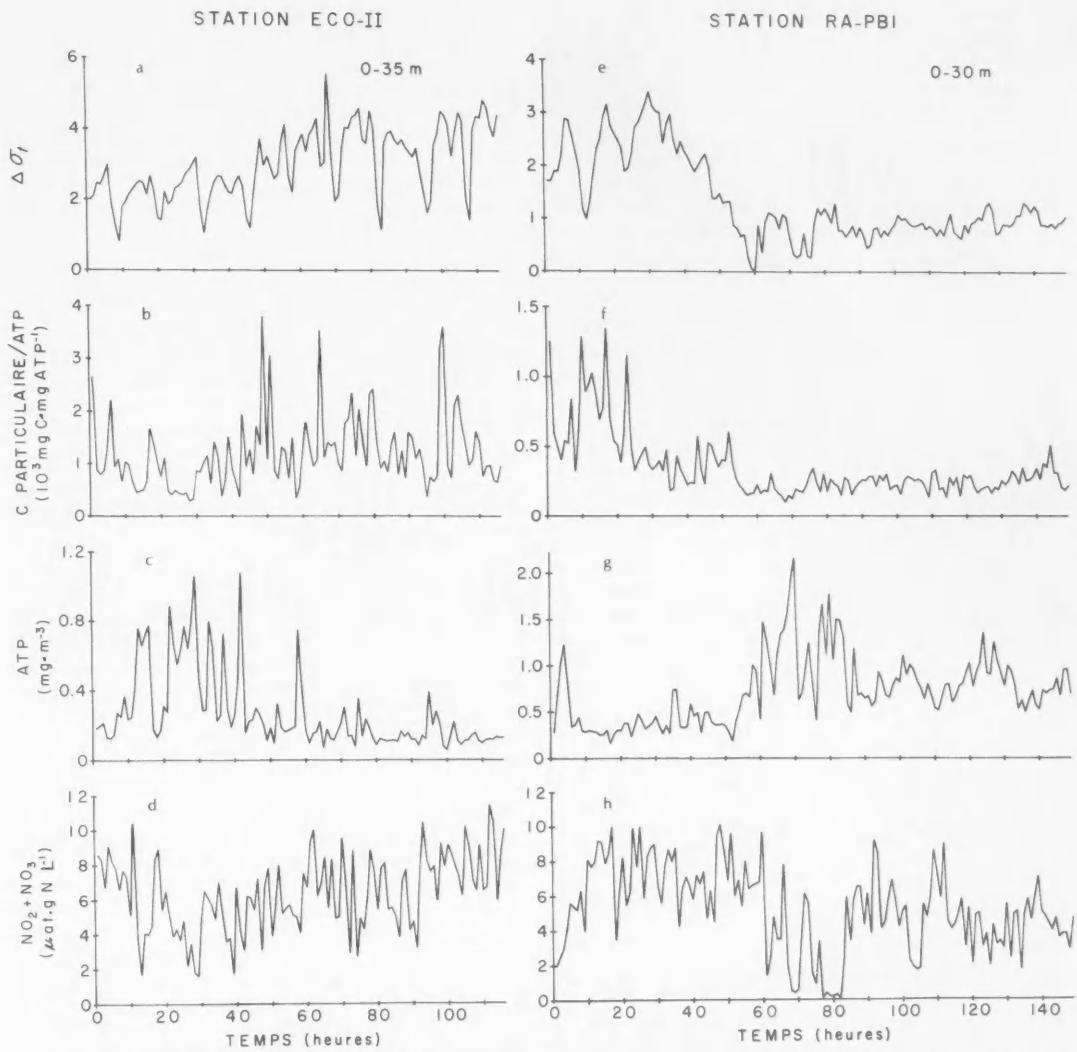

FIG. 3. Evolution horaire du gradient vertical de densité ($\Delta\sigma_t$: a, e), du rapport carbone particulaire/ATP (b, f), de la biomasse (ATP: c, g) et des nitrates ($NO_2 + NO_3$: d, h) aux stations ECO-II et RA-PBI de l'estuaire maritime du Saint-Laurent.

marée. La même rythmicité existe probablement dans la portion maritime de la série ($t = 0$ à 24 h) mais se trouve partiellement masquée par la superposition de variations importantes et non cycliques.

VARIABLES BIOLOGIQUES ET CHIMIQUES

Les indices de biomasse totale (ATP particulaire) (fig. 3c) et de biomasse photosynthétique (chlorophylle *a*) (fig. 4d) varient de façon remarquablement similaire. Les coefficients de corrélation élevés entre ces deux variables aux deux stations ($\tau = 0.564$ à ECO-II et 0.641 à RA-PBI, $P < 0.01$) suggèrent que la matière vivante mesurée était essentiellement phytoplanctonique. Les valeurs sont en accord avec celles fournies par Sinclair (1978) pour ces deux régions de l'Estuaire maritime, au mois de juillet 1975. A la station ECO-II, la production primaire mesurée en lumière saturante est en forte ($P < 0.01$) corrélation (τ) positive avec la biomasse présente (ATP et chlorophylle *a*).

Dans les eaux superficielles, aux deux stations, il existe une forte corrélation inverse ($P < 0.01$) entre

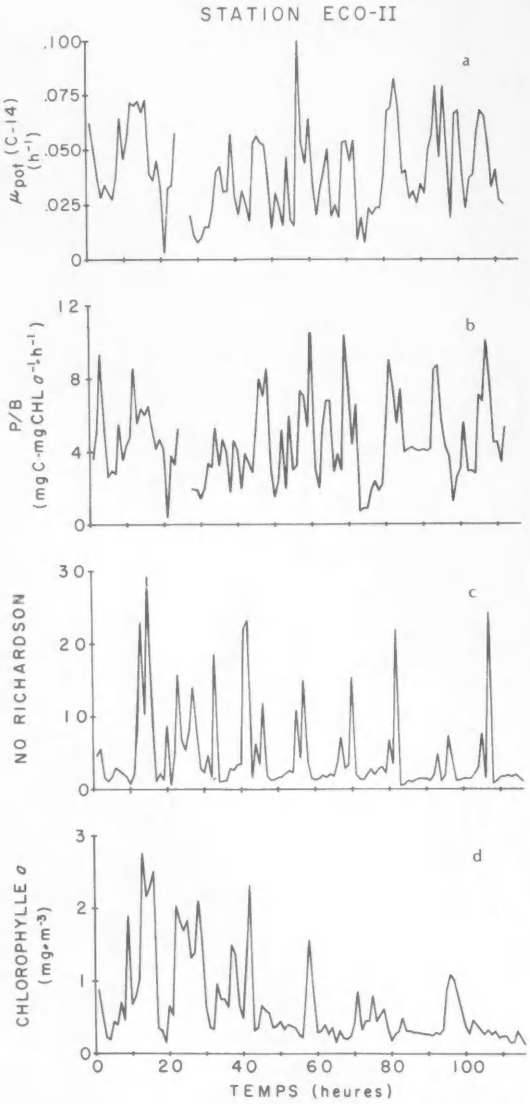

FIG. 4. Evolution horaire à la station ECO-II (20–25 juillet 1977) de l'estuaire maritime du Saint-Laurent. (a) taux de croissance potentiel calculé à partir de l'assimilation du ^{14}C ($\mu_{pot(^{14}C)}$); (b) rapport P/B; (c) stabilité (nombre de Richardson); (d) chlorophylle *a*.

la biomasse (ATP et chlorophylle *a*) et la concentration de nitrates (fig. 3c, 3d, 3g et 3h). En présence de biomasse élevée, les nitrates peuvent parfois atteindre des niveaux pratiquement indétectables (fig. 3h: $t = 60$ à 80 h). Simultanément, on enregistre une diminution des phosphates, qui n'atteignent cependant ja-

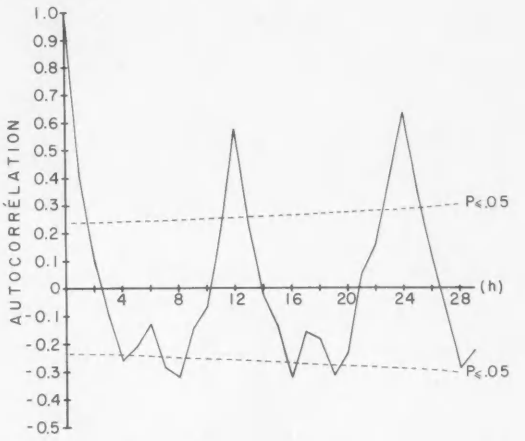

FIG. 5. Corrélogramme de la série horaire du rapport P/B, mesuré à la station ECO-II ($n = 72$) en régime continental. Les intervalles de confiance correspondent à un niveau de probabilité de 5%.

mais un niveau susceptible d'entraîner une limitation de la photosynthèse. Cette prépondérance des nitrates comme élément nutritif potentiellement limitant en milieu estuarien a déjà été notée par Winter et al. (1975), pour le fjord de Puget Sound.

L'évolution horaire de $\mu_{pot(^{14}C)}$ et de P/B (fig. 4a et 4b) présente certaines particularités qui peuvent être associées à des variations du milieu physique. On note ainsi à partir de la 42ème heure, soit au passage du con-

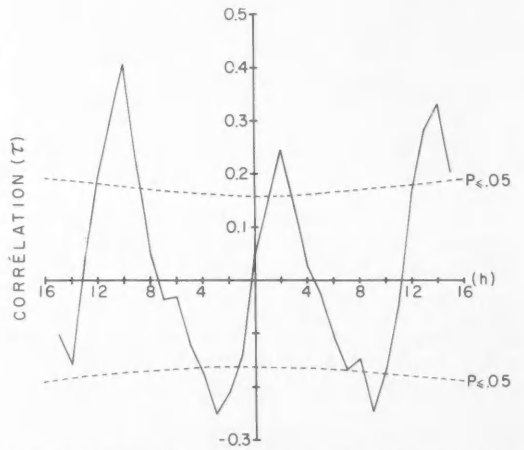

FIG. 6. Corrélation de Kendall avec retard (τ) entre l'évolution horaire du rapport P/B et celle de la stabilité ($\hat{R}i$), à la station ECO-II ($n = 72$) en régime continental. Les intervalles de confiance correspondent à un niveau de probabilité de 5%.

texte maritime au contexte fluvial, que P/B et $\mu_{pot(^{14}C)}$ présentent une nette rythmicité semi-diurne avec des maxima au flot du cycle de marée. Le corrélogramme (fig. 5) pour cette portion des séries indique que cette rythmicité est significative ($P < 0.05$) pour les deux indices et que sa période est d'environ 12 h. Etant donnée la faible longueur de la série, une telle période de 12 h masque la présence d'une éventuelle périodicité photosynthétique circadienne.

Réponse phytoplanctonique aux variations physiques

A la station ECO-II, la biomasse (ATP et chlorophylle *a*) et la production primaire sont en corrélation (τ) inverse ($P < 0.01$) avec la stratification verticale de la colonne d'eau, $\Delta\sigma_t$. En ce qui a trait à la biomasse, ces résultats sont confirmés à la station RA-PBI. L'allure générale des courbes (fig. 3a, 3c, 3e et 3g) révèle clairement la limite entre les régimes maritimes et fluviaux, soit à la 42ème heure à ECO-II et à la 54ème heure à RA-PBI.

L'évolution temporelle horaire de P/B, de la stabilité verticale de la colonne d'eau ($\hat{R}i$) et de la biomasse (chlorophylle *a*) sont comparées à la fig. 4, pour la station ECO-II. Dans la portion maritime de la série ($t = 0$ à 42 h), la biomasse est fortement liée à la stabilité verticale du milieu ($\tau = 0.480$, $n = 42$, $P < 0.01$) alors que P/B ne semble pas en relation monotone avec ce dernier paramètre. La portion continentale de la série ($t = 42$ à 116 h) présente des résultats opposés. La très faible biomasse ne semble pas distribuée en fonction de la stabilité verticale, cependant les maxima de P/B sont systématiquement associés à des pics de stabilité. En fait, la corrélation de Kendall avec retard (fig. 6) indique que l'évolution des deux variables n'est pas parfaitement synchrone, mais qu'une forte activité photosynthétique se poursuit pendant environ deux heures, en moyenne, après la disparition de conditions stables dans la colonne d'eau. Des relations similaires sont décelées lorsqu'on utilise l'ATP et $\mu_{pot(^{14}C)}$ comme indices de biomasse et d'activité photosynthétique respectivement.

Les spectres de variance de la température et du nombre de Richardson (stabilité verticale) sont présentés à la fig. 8. La gamme des fréquences résolues s'étend de 0.047 cycles·h^{-1} (T = 21.33 h) à 24 cycles·h^{-1} (T = 2.5 min). Les échelles de densité de variance correspondent aux valeurs réelles et chaque point du spectre est constitué de la moyenne de 8 sous-estimations.

Discussion

Stratification et turbidité

Dans l'estuaire maritime du Saint-Laurent, la rythmicité semi-mensuelle que l'on observe dans l'évolution estivale de la stratification a été interprétée par Sinclair (1978) comme une conséquence des variations de l'intensité du mélange. Une autre hypothèse plausible est celle de l'envahissement périodique des eaux de surface de l'Estuaire maritime par des apports fluviaux plus importants en provenance de l'Estuaire moyen inférieur, reconnu pour la turbidité et la pauvreté en phytoplancton de ses eaux (Cardinal et Lafleur 1977; Fortier et al. 1978; Lafleur et al. 1979). La périodicité du phénomène serait alors contrôlée par l'importance de l'intrusion saline qui dépend, elle aussi, du terme semi-mensuel de marée M_f.

L'augmentation brusque de la stratification (fig. 3a et 3e) et du rapport carbone particulaire/carbone vivant (fig. 3b et 3f) lors de la transition d'un régime maritime à un régime continental suggèrent un accroissement de la turbidité imputable à une augmentation, en surface, du matériel particulaire non vivant et à une réduction du taux de sédimentation. Les travaux de Sinclair (1978) dans la même région ont d'ailleurs démontré que, à l'échelle d'une semaine, la distribution estivale de la turbidité des eaux de surface est similaire à celle du gradient vertical de densité. La réponse phytoplanctonique consiste en une diminution très nette de la biomasse moyenne (fig. 3c et 3g). Ces résultats, obtenus à partir d'un échantillonage horaire et vérifiés aux deux stations occupées, sont en accord avec ceux de Sinclair (1978), obtenus à partir de prélèvements hebdomadaires.

Stabilité

Dans un milieu estuarien stratifié comme l'estuaire martime du Saint-Laurent en été, la source prépondérante de brassage vertical est probablement la turbulence créée à l'interface des couches d'eau se déplaçant à des vitesses différentes. A court terme, l'évolution horaire du nombre de Richardson (fig. 4c), malgré les imprécisions inhérentes à son calcul, donne vraisemblablement une image assez exacte de la stabilité relative de la colonne d'eau en fonction du temps. Il est évident que, en l'absence de données suffisantes pour calculer de façon exacte les gradients verticaux de densité et de vitesse relative ($\delta\rho/\delta z$ et $\delta v/\delta z$), les valeurs absolues de $\hat{R}i$ possèdent peu de signification sur le plan strictement physique.

Dans un estuaire, le processus de stabilisation peut être visualisé comme un mascaret interne se déplaçant vers l'amont, de la même façon que l'onde de marée dont il dépend. Entre Pointe-au-Père et Rivière-du-Loup (fig. 1) la célérité de l'onde de marée, et conséquemment celle de cette « onde de stabilisation », est en moyenne d'environ 123 km·h^{-1} (Partenscky et Warmoes 1970). Par rapport à une point d'observation fixe (la station d'échantillonnage), l'effet du déplacement vers l'aval de la masse d'eau (2.5 km·h^{-1}) dans laquelle l'onde se propage vers l'amont, est donc négligeable.

A la station ECO-II, l'évolution horaire du nombre de Richardson (fig. 4c) présente effectivement, en ré-

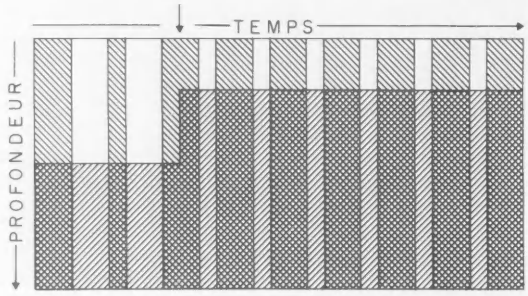

FIG. 7. Schéma du modèle d'interaction temporelle et verticale entre la stabilité et la profondeur critique à la station ECO-II.\\\\: zones de faible stabilité verticale; ////: zones où l'intensité lumineuse est insuffisante pour la croissance (production $\leq$ respiration). Les zones vierges représentent les domaines spatio-temporels où la croissance du phytoplancton est possible. La flèche indique le passage d'un régime maritime à un régime continental. (Voir le texte).

gime continental, un cycle semi-diurne lunaire dont la période de 12.5 h se subdivise en une période de stabilité d'environ 4 h ($\hat{R}i > 3$) et en une période de brassage vertical potentiel d'environ 8 h ($\hat{R}i$ faible). En régime marin, l'onde de stabilisation observée semble se déformer de façon à produire des périodes plus longues de stabilité. En tenant compte de la durée de ces périodes de stabilité ou de brassage vertical et de la vitesse de propagation de l'onde, on peut conclure que les conditions mesurées à la station, à un quelconque moment du cycle de marée, représentent un phénomène généralisé sur le plan spatial.

Les résultats indiquent que, lorsqu'il n'est pas limité par la turbidité (régime maritime), le développement en surface d'hétérogenéités dans la distribution temporelle de la biomasse phytoplanctonique est intimement lié à la stabilité de la colonne d'eau, c'est-à-dire, à l'absence de brassage vertical (e.g. fig. 4: $t = 11$ à 16 h et $t = 21$ à 29 h). Le brassage vertical au contraire, par l'effet combiné de la diffusion et de la réduction du temps de résidence due à l'entraînement du phytoplancton sous la couche photique, réduit de façon draconienne la biomasse en surface.

L'examen de la portion continentale (plus turbide) de la série semble confirmer le rôle de la croissance phytoplanctonique dans le processus. En effet, les résultats montrent que les maxima périodiques d'activité photosynthétique (P/B) sont associés à des pics de stabilité. En milieu turbide, la stabilité déclencherait donc l'activité photosynthétique du phytoplancton de surface, mais la brièveté du phénomène et la couche photique diminuée ne semblent pas permettre le développement d'une biomasse importante.

Il est à noter que le mécanisme proposé est analogue à celui de la profondeur critique (Sverdrup 1953). Le travail plus récent de Fournier et al. (1979) propose

TABLEAU 1. Taux de croissance théoriques calculés à partir de l'évolution temporelle de la chlorophylle *a* ($k_{\text{Chl } a}$) et de l'ATP (k_{ATP}) et taux de croissance potentiels mesurés en incubateur par l'assimilation du ^{14}C ($\bar{\mu}_{\text{pot}(^{14}C)}$) et par la production d'ATP ($\bar{\mu}_{\text{pot}(\Delta\text{ATP})}$). Les taux de croissance sont en h^{-1}.

	k (Chl *a*)	k (ATP)	$\bar{\mu}_{(\text{pot})}$ (^{14}C)	$\bar{\mu}_{\text{pot}}$ (ΔATP)
	0.18	0.15	0.05	0.12
Max.	—	—	0.07	0.22
Min.	—	—	0.03	0.01

un mécanisme similaire pour expliquer la relation inverse entre la distribution spatiale de la stabilité et celle de la chlorophylle, sur le plateau continental de la Nouvelle-Ecosse.

Pour la station ECO-II, l'évolution temporelle de l'interaction entre la stabilité, qui détermine la profondeur de mélange, et la profondeur critique, a été schématisée à la fig. 7. Les zones non hachurées représentent les domaines spatio-temporels où sont réunies simultanément la stabilité verticale de la colonne d'eau et l'intensité lumineuse suffisante pour la croissance du phytoplancton. Ces zones sont limitées, sur l'axe temporel, par la durée des périodes de stabilité et, sur l'axe vertical, par la profondeur de la couche photique. En régime marin, les surfaces ainsi délimitées sont en général plus grandes qu'en régime continental, ce qui expliquerait la différence observée entre les deux régimes hydrauliques, quant à l'importance de la réponse phytoplanctonique.

TEMPS DE RÉPONSE DU PHYTOPLANCTON

Régime maritime — Puisque les conditions de stabilité mesurées à la station représentent un phénomène généralisé sur le plan spatial, on peut supposer que la composante temporelle de leur variabilité soit prépondérante. En régime maritime, ceci implique que les développements phytoplanctoniques observés lors de la stabilisation de la colonne d'eau se produisent essentiellement à l'intérieur de ces périodes de stabilité relative. La diminution des nitrates associée aux fortes biomasses observées en régime maritime, de même que le comportement du rapport P/B et de $\mu_{\text{pot}(^{14}C)}$ en régime continental, suggèrent que les processus de croissance sont directement impliqués dans cette augmentation de la biomasse et qu'il ne s'agit pas uniquement de ces phénomènes d'advection verticale discutés par Smayda (1970) et décrits par Ganf (1974). Ces constatations confirment, pour l'axe temporel, l'hypothèse sugérée par Platt (1975) et par Denman et Platt (1975) puis vérifiée par Therriault et al. (1978) pour l'axe spatial, selon laquelle l'hétérogénéité qu'on observe dans la distribution de la biomasse phytoplanctonique est la résultante de l'équilibre entre les processus biologiques et les processus turbulents.

Dans ces processus de croissance, la biomasse initiale serait, en théorie, du même ordre de grandeur que la biomasse mesurée lors des périodes de mélange vertical qui précèdent les périodes de stabilité, alors que la biomasse atteinte pendant les périodes de stabilité constituerait la biomasse finale. Cependant, dans un estuaire, la détermination exacte du taux de croissance à partir de l'évolution temporelle de la biomasse s'avère hasardeuse puisque la distribution spatiale de la biomasse initiale ne peut être supposée uniforme. Par rapport à la station d'échantillonnage, cette hétérogénéité horizontale se manifeste en fonction du déplacement des masses d'eau lié aux courants de marée (Fortier et al. 1978).

En régime maritime, les augmentations en quelques heures de la biomasse par un facteur de 5 suggèrent une croissance exponentielle du phytoplancton. L'examen des fig. 4c et d montre qu'effectivement l'augmentation de la biomasse associée aux périodes de stabilité moyenne ($\hat{R}i \simeq 3$), qui précèdent les périodes de stabilité maximale ($\hat{R}i > 20$), présente une allure approximativement exponentielle malgré certaines irrégularités.

Supposant que la croissance du phytoplancton soit exponentielle, on peut calculer le taux de croissance théorique (k) nécessaire au développement des structures phytoplanctoniques (ou "taches") rencontrées:

$$k = (1/t) \ln (B_t/B_o)$$

où t représente la durée (en heures) de la période apparente de croissance exponentielle, B_o, la biomasse moyenne au cours du brassage vertical précédant la période de stabilité et B_t, la biomasse finale atteinte en conditions de stabilité maximale. Ce taux de croissance théorique a été calculé pour la période d'augmentation exponentielle de la biomasse (fig. 3c et 4d: $t = 4$ à 13 h), observée en régime maritime à la station ECO-II, immédiatement avant la première structure phytoplanctonique bien définie ($t = 13$ à 16 h). Les valeurs obtenues à partir de l'évolution temporelle de la chlorophylle et de l'ATP sont présentées à titre purement indicatif (Tableau 1) aux fins de comparaison avec la moyenne des taux de croissance potentiels ($\bar{\mu}_{pot}$) pour les 16 premières heures de prélèvement. Les taux de croissance théoriques (k), calculés à partir de l'évolution temporelle de la chlorophylle et de l'ATP sont relativement similaires. On remarque aussi que l'estimation, en incubateur, du taux de croissance potentiel moyen par la production d'ATP ($\bar{\mu}_{pot(\Delta ATP)}$) est de quelque deux fois supérieure à celle obtenue en utilisant l'assimilation du ^{14}C ($\bar{\mu}_{pot(^{14}C)}$) comme indice de l'augmentation du carbone. Une telle divergence entre les deux méthodes a récemment été notée et discutée par Sheldon et Sutcliffe (1978) pour un milieu tropical.

Les résultats du tableau 1 montrent, de plus, que l'estimation du taux de croissance potentiel moyen à partir de l'ATP ($\bar{\mu}_{pot(\Delta ATP)}$) est du même ordre de grandeur que les taux de croissance théoriques (k) calculés à partir de l'évolution temporelle de la biomasse, surtout si on tient compte du maximum que peut atteindre $\bar{\mu}_{pot(\Delta ATP)}$. Ce résultat suggère que la productivité potentielle des populations rencontrées en régime maritime pourrait être suffisante pour expliquer, en termes de croissance, les développements phytoplanctoniques associés à la stabilisation de la colonne d'eau.

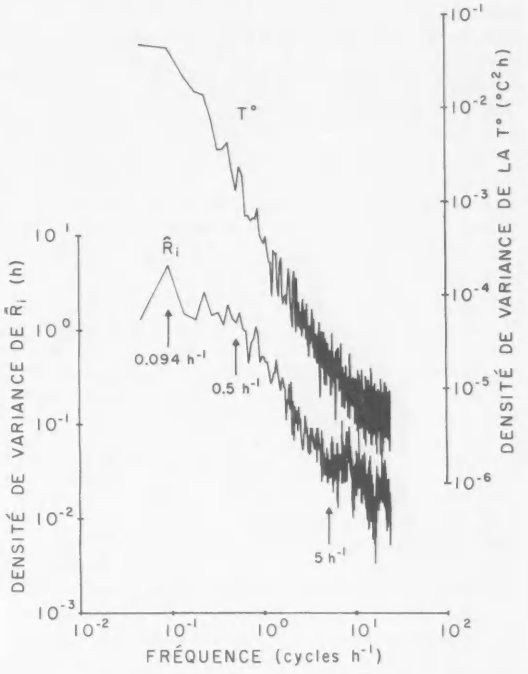

FIG. 8. Spectres lissés de la densité de variance du nombre de Richardson et de la température, à la station ECO-II. Le nombre de Richardson étant non dimensionnel, sa densité de variance d'exprime en heures.

Régime continental — En régime continental, le décalage d'environ deux heures (±1 h) entre l'évolution du nombre de Richardson et celle du rapport P/B (fig. 6) reflète la persistance de l'activité photosynthétique après l'apparition du brassage vertical qui succède aux périodes de stabilité. L'examen attentif des figures 4b et 4c suggère en effet que, bien que le début de l'augmentation du rapport P/B soit synchronisé à l'augmentation de la stabilité, les fortes valeurs persistent après la diminution du nombre de Richardson, c'est-à-dire après le début du brassage vertical. Il semble donc qu'une certaine période (2 h ± 1 h) de conditions physiques défavorables soit nécessaire pour ralentir l'activité photosynthétique induite par la stabilisation de la colonne d'eau.

ANALYSE SPECTRALE

Le mécanisme proposé plus haut, pour expliquer le contrôle de la biomasse phytoplanctonique par la stabilité de la colonne d'eau, prédit que

les distributions temporelles de ces deux variables coïncident, dans des conditions de turbidité relativement constantes et non limitantes. Dans la présente étude, le nombre relativement faible de prélèvements biologiques (116 prélèvements horaires) ne permet pas l'utilisation de l'analyse spectrale sur les séries de biomasses. Par ailleurs, les données physiques ayant été échantillonnées à intervalles de 1.25 min pendant 173 h (14 cycles semi-diurnes de marée), les séries temporelles résultantes se prêtent relativement bien à l'analyse spectrale.

Le spectre de variance de la distribution temporelle de la température (fig. 8) est semblable à celui décrit par Dyer (1973, p. 27) pour l'énergie turbulente en milieu estuarien. La densité maximale de variance se retrouve aux fréquences de 0.047 cycle·h^{-1} (T = 21.33 h) et 0.094 cycle·h^{-1} (T = 10.66 h), qui sont les fréquences résolues par l'analyse spectrale se rapprochant le plus de celle du cycle semi-diurne de marée (M_2). Le transfert de cette variance, des structures temporelles de grande dimension (>0.094 cycle·h^{-1}) vers les structures de dimensions réduites (≃5 cycles·h^{-1}) se fait selon un processus de cascade similaire à celui décrit par Kolmogorov (1941).

Pour le nombre de Richardson, la densité de variance maximale apparaît aussi à une fréquence de 0.094 cycle·h^{-1} (fig. 8), ce qui correspond à la structuration de la stabilité induite par le cycle semi-diurne de marée (fig. 4c). Cependant, des densités élevées de variance sont observées jusqu'à des échelles plus petites que pour la température, le transfert par un processus de cascade vers les structures de dimensions réduite (≃5 cycles·h^{-1}) ne s'effectuant qu'à partir d'une fréquence d'approximativement 0.5 cycle·h^{-1} (T = 2 h).

Selon le mécanisme proposé dans le présent article pour expliquer, en régime maritime, la relation entre la stabilité et la biomasse photosynthétique, l'inflexion que l'on observe à cette fréquence (0.5 cycle·h^{-1}) dans le spectre de $\hat{R}i$ devrait également se produire dans le spectre de la distribution temporelle de la biomasse, lequel n'est malheureusement pas disponible. Une telle rupture de pente a cependant déjà été observée par Denman (1976) et Denman et Platt (1975) dans le spectre de la distribution spatiale de la chlorophylle *a*, pour le secteur inférieur de l'Estuaire maritime où la turbidité n'est vraisemblablement pas limitante.

Les spectres observés sont donc en accord avec l'hypothèse formulée au début de la discussion. Ils permettent de plus de rattacher à ce modèle simple les observations sur les spectres de la chlorophylle en milieu aquatique, et notamment la présence d'une cassure ("knee": Platt 1975; Denman et Platt 1975; Denman 1976; Fasham et Pugh 1976 et Demers et al. 1979) aux fréquences intermédiaires. Selon cette hypothèse, les variations temporelles de basse fréquence de la stabilité amplifieraient l'hétérogénéité spatiale d'un paramètre actif (reacting field: Corrsin 1961) comme la chlorophylle, alors qu'un contaminant passif du milieu comme la température répondrait de façon moins évidente (fig. 8) à de telles variations. Il n'est donc pas surprenant, dans les travaux antérieurs cités ci-dessus, d'observer une divergence entre les spectres de la température et de la chlorophylle jusqu'aux échelles intermédiaires. De ce fait, les études sur le contrôle physique du phytoplancton se sont limitées jusqu'à présent aux fréquences intermédiaires, la divergence aux basses fréquences étant attribuée à l'activité biologique. L'examen du spectre de stabilité ($\hat{R}i$) permet d'étendre aux basses fréquences l'étude du contrôle physique de la dynamique phytoplanctonique puisque, à ces échelles, les processus biologiques eux-mêmes repondent aux fluctuations du milieu physique.

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Examination of Phosphorus Cycling and Control of Phytoplankton Dynamics in Lake Ontario with an Ecological Model[1]

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SCAVIA, D. 1979. Examination of phosphorus cycling and control of phytoplankton dynamics in Lake Ontario with an ecological model. J. Fish. Res. Board Can. 36: 1336–1346.

An ecological model of Lake Ontario was used to assist in interpretation of data collected during the International Field Year for the Great Lakes (March 1972–April 1973). The analysis indicated that in spring and fall phytoplankton biomass is controlled by the interaction of incoming solar radiation and vertical mixing, in summer by silica- and phosphorus-limitation, and in late summer by zooplankton grazing. The influence of $CaCO_3$ precipitation on the light climate in late summer was also demonstrated. During the period of stratification, available phosphorus concentration is controlled by recycling within the epilimnion, primarily through plant and animal excretion. Comparison of simulated available phosphorus concentrations and concentrations of total dissolved phosphorus and soluble reactive phosphorus in the epilimnion suggest that the composition of the soluble unreactive phosphorus pool changes dramatically during the year and that the large pool of dissolved unavailable phosphorus during summer is composed of end products of material cycled several times through the food web.

Key words: phytoplankton dynamics, phosphorus cycle, Lake Ontario, ecological model, nutrient recycling, phytoplankton production

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A l'aide d'un modèle écologique du lac Ontario, nous interprétons les données recueillies durant l'Année internationale d'étude des Grands Lacs (mars 1972–avril 1973). On constate à l'analyse que la biomasse phytoplanctonique de printemps et d'automne est réglée par l'interaction de la radiation solaire incidente avec le mélage vertical, en été par limitation de la silice et du phosphore et à la fin de l'été par le broutage du zooplancton. Nous démontrons aussi l'influence de la précipitation de $CaCO_3$ sur le climat lumineux en fin d'été. Durant la période de stratification, la concentration de phosphore disponible est réglée par recyclage dans l'épilimnion, surtout par excrétion végétale et animale. Une comparaison des concentrations de phosphore disponible simulées et de celles de phosphore dissous total et de phosphore réactif soluble dans l'épilimnion donne à penser que la composition du pool de phosphore non réactif soluble change de façon dramatique durant l'année et que le grand pool de phosphore non disponible dissous en été est composé des produits finals d'un matériel plusieurs fois recyclé dans le réseau alimentaire.

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LAKE Ontario, the lowermost lake in the chain of Laurentian Great Lakes, has experienced considerable stress over the past several decades. Among the Great Lakes, the rate of eutrophication in Lake Ontario has been surpassed only by that in Lake Erie (Beeton 1965, 1969). It is important to understand the overall ecology of Lake Ontario to assess its present condition, to evaluate trends in its trophic status, and to suggest actions that might be taken to restore and protect this precious water body.

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By necessity, a lake the size of Lake Ontario has been studied through discrete and often isolated research programs. Over the past decade, several excellent studies have been conducted illuminating the controls and mechanisms of several important ecological and physical processes. For example, there have been studies on primary production and phytoplankton turnover rates (e.g. Glooschenko et al. 1974; Stadelmann and Munawar 1974; Munawar et al. 1974; Stadelmann et al. 1974); diurnal migrations of crustaceans (Wilson and Roff 1973); phosphorus and nitrogen cycling (Stadelmann and Fraser 1974); vertical transport of heat (Sweers 1970) and of carbon and phosphorus (Burns and Pashley 1974); grazing by zooplankton (Gloos-

chenko et al. 1972); large-scale horizontal diffusion (Kullenberg et al. 1973) and current regimes (Pickett and Richards 1975; Pickett 1976a, 1977; Pickett and Bermick 1977); and benthic community metabolism (Johnson and Brinkhurst 1971). However, there have been few attempts to examine simultaneously several of these processes through field or laboratory experiments. Where combinations of processes have been examined, it has been done, by necessity, for only one or two stations (e.g. Lean et al. 1975; Lean and Charlton 1976). Information from these studies is important for understanding ecosystem controls; however, because Lake Ontario is neither homogenous horizontally nor simple hydrodynamically, it is not possible to extrapolate information from one or two stations to estimate lakewide conditions as, for example, Jassby and Goldman (1974) were able to do for Castle Lake, Calif. Thus, a comprehensive view of whole-lake processes for Lake Ontario has not been possible.

Because it is unlikely that a large field program designed to investigate important processes on a lakewide scale is tractable for Lake Ontario, some other method of synthesizing the available information and of processing that information to generate insight into whole-lake processes is needed. One method of synthesis is to summarize available information on Lake Ontario, to intuit qualitatively the interactions among ecosystem components, and to attempt to deduce the relative importance of specific processes where information on those processes exists. Another method is to develop some mathematical representation of the ecosystem and the interactions among its components, and thereby to produce some quantitative, yet theoretical, information on the processes. The method employed in this work was to use a model of the Lake Ontario ecosystem, based on theoretical concepts of nutrient cycles and food web ecology, together with data collected on Lake Ontario to combine the above two approaches.

Output from the model can never supplant information derived from experimental and observational programs; however, because the model simultaneously processes information from several interacting ecosystem components, it provides a larger, more systematic framework than is usually available from isolated experiments. By examining the processes that control a certain component of the model ecosystem, one can evaluate the magnitude and relative importance of these processes on a seasonal basis. Also, hypotheses concerning certain processes that are not presently understood very well can be tested. In essence, the model is merely a testing ground for hypotheses. As Steele (1974) has suggested, the model does not create any new information, but rather it forces a quantitative analysis of the system as a whole. This analysis very often results in identification of important areas requiring further experimental work, which then brings coupled modeling-experimental programs (see Mortimer 1975) around full circle. The model, designed originally to test hypotheses generated by experimental and observational studies, can then be used to generate further hypotheses that must be tested experimentally.

By using this approach of combined data analysis and mathematical modeling, three aspects of the Lake Ontario ecosystem were examined: (1) the factors controlling the dynamics of phytoplankton biomass, (2) the seasonal dynamics of available phosphorus and the relationship of these dynamics to control of phytoplankton production, and (3) the apparent changes in chemical composition of various phosphorus compartments.

Model and Data

Empirical relationships (e.g. Vollenweider 1968; Dillon and Rigler 1974a) and mass balance models (e.g. Dillon and Rigler 1974b; Imboden 1974; Chapra 1977), developed to relate gross properties in lakes, are useful in describing or predicting long-term trends in water quality. However, these models fall short of providing much insight into the interrelationships within, and the control of, the complex aquatic ecosystems they represent (Shapiro 1978). Therefore, if one wishes to use models to investigate processes within the ecosystem, then other, more realistic models must be used.

More mechanistic models exist for Lake Ontario. Thomann et al. (1975) developed a eutrophication model based on phytoplankton dynamics for the purpose of estimating the impact of variations in nutrient loads. The model was applied in that context to assess various management alternatives (Thomann et al. 1977). Simons (1976) also used the model, within a three-dimensional hydrodynamic framework, to examine the influences of physical processes on the simulation of phytoplankton dynamics. Concurrently, another model was developed to simulate the flow of carbon, nitrogen, and phosphorus through the food web of Lake Ontario (Scavia et al. 1976a). The generality of the framework of this model was tested through applications to the other Great Lakes (Scavia et al. 1976b) and by comparison with output from some of the simpler models mentioned above (Scavia and Chapra 1977). The model used herein is an extension of this latter model.

The purpose of the model is to synthesize information on process kinetics into equations describing the rate of change of ecosystem components as functions of the components themselves, of environmental variables, and of group-specific coefficients. Process equations posed by experimentalists over the past several years are used to describe specific biological and chemical processes. Granted, for some of the processes more than one theory or hypothesis has been espoused by researchers as a control or regulator. Use of a particular process equation in the overall fromework is one way to test that theory or hypothesis in the context of the larger system. As such, the model is merely a tool allowing a quantitative evaluation of isolated process theories in a whole-system context.

The model (Fig. 1) includes phytoplankton; zooplankton; cycles of phosphorus, nitrogen, silicon, and carbon; an oxygen balance; and calculations of the carbonate equilibrium system. A sediment compartment includes benthic invertebrates as well as cycles of carbon, phosphorus, nitrogen, and silicon. The reason for segregating phytoplankton and zooplankton each into several groups is to allow for differences in functional requirements (e.g.

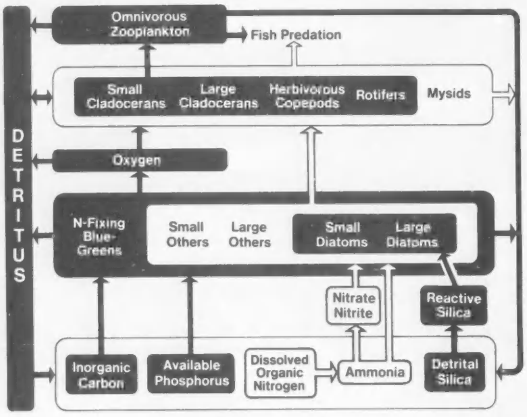

FIG. 1. Conceptualization of Lake Ontario ecological model.

diatoms versus nondiatoms) and processes (e.g. size-selective filter feeders versus nonselective feeders). The major species of phytoplankton and zooplankton in Lake Ontario and their aggregation into functional groups are listed in Table 1.

Each model compartment is represented by a differential equation composed of important biological, chemical, and physical processes. For example, the phytoplankton equation includes terms for gross primary production, respiration, excretion, grazing, sinking, and vertical mixing, and the zooplankton equation includes terms for grazing, assimilation, respiration, excretion, defecation, and predation. Justification and rationales for the process equations used, as well as detailed documentation of the overall model are presented in Scavia (1979). What follows are general descriptions of the processes included in the model.

Gross phytoplankton production is considered a single-step process, which assumes, for the time-scale of the model (several days), that uptake and growth rates are equal. The process is modeled with a temperature-dependent, maximum growth rate times a reduction factor for light- and nutrient-limitation. Potential light-limitation is modeled after Vollenweider (1965) and Steele (1965) and potential nutrient-limitation is expressed as a Michaelis–Menten term for each nutrient. The threshold formulation is used to determine overall limitation. Phytoplankton respiration is considered as the sum of a low maintenance rate plus a term proportional to production, as well as being a function of temperature. To maintain constant nutrient stoichiometry within the plankton, nutrient excretion is proportional to respired carbon.

Zooplankton grazing is handled as a temperature-dependent Michaelis–Menten expression based on total food supply. The expression accounts for minimum thresholds for feeding as well as resource partitioning based on size-selection. Feeding and assimilation efficiency are regarded as food-specific constants. Food ingested but not assimilated is egested into the detritus pool. Respiration is a temperature-dependent process that is the sum of a low maintenance rate and a term proportional to the feeding rate. Nutrient excretion is proportional to respiration.

Transformation rates between detritus, dissolved organic nitrogen, ammonia, nitrate plus nitrite, available phosphorus, and available silicon pools all are temperature-dependent and first-order. Sinking rates of phytoplankton and detritus are size- and density-dependent. Vertical mixing is modeled as an exchange coefficient (see below) times the concentration gradient.

The model considers the lake to be homogeneous in the horizontal plane and to be segmented vertically into two layers representing the lake-wide average epilimnion and hypolimnion. A 1-dimensional, 18-layer diffusion model calibrated to temperature profiles measured in Lake Ontario is used to calculate depth of the thermocline and average epilimnion and hypolimnion temperatures. Values of the diffusion (or exchange) coefficient between the two layers are then calculated from temperature changes in

TABLE 1. Lake Ontario phytoplankton and zooplankton functional groups used in the ecological model[a]

Small Diatoms	**Small Cladocerans**
Melosira islandica	*Eubosmina coregoni*
Stephanodiscus alpinus	*Bosmina longirostris*
S. binderanus	
S. hantzschii	**Large Cladocerans**
S. minutus	
S. subtilis	*Daphnia galeata*
S. tenuis	*D. retrocurva*
	D. longiremis
Large Diatoms	*Ceriodaphnia lacustris*
	Chydorus sphaericus
Asterionella formosa	*Diaphanasoma leuchtenbergianum*
Diatoma tenue var. *elongatum*	
Fragilaria capucina	**Herbivorous Copepods**
F. crotonensis	
Nitzschia bacata	*Calanoid copepodites*
N. sp.	*Diaptomus minutus*
Surirella angusta	*D. oregonensis*
Taballaria fenestrata	*D. sicilis*
	D. siciloides
Small Others	*Eurytemora affinis*
Flagellate #1 F	**Carnivores/Omnivores**
Glenodinium and *Gymnodinium* spp. Dn	*Cyclopoid copepodites*
Gloecystis planctonica G	*Cyclops biscuspidatus*
Oocystis spp. G	*C. vernalis*
Oscillatoria limnetica B	*Tropocyclops prasinus*
Phacotus lenticularis G	
Scenedesmus bicellularis G	**Blue-Green Algae with Heterocyst**
Large Others	*Anabaena flos-aquae*
Cryptomonas erosa Cr	*A.* sp. #1
Peridinium spp. Dn	*A. variabilis*
Staurastrum paradoxum G	
Ulothrix subconstrictun G	

[a]Algae other than blue-greens are only those whose annual average concentration is greater than 0.5 μg C/L (Stoermer and Ladewski 1978). Symbols are: B — blue-green without heterocyst; G — green; F — flagellate; Dn — dinoflagellate; Cr — cryptomonad. Zooplankton species from McNaught et al. (1975).

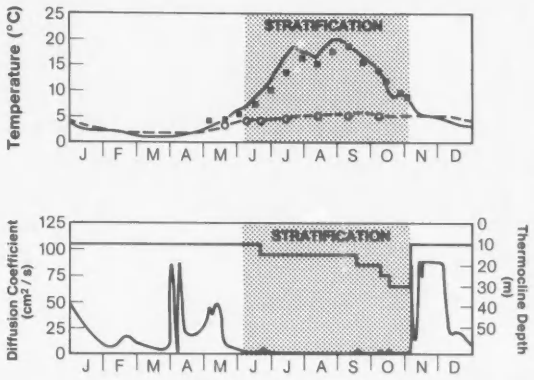

FIG. 2. Simulated and observed (Pickett 1976b) lakewide averaged temperatures. (*Top*): ■ — epilimnion, ○ — hypolimnion. Simulated thermocline depth and diffusion coefficients (*bottom*).

the two layers and from the extent of thermocline displacement (Fig. 2).

The ecological model was calibrated with data collected during the International Field Year for the Great Lakes (IFYGL) from March to November 1972. Documentation of the seasonal cycles of the IFYGL data and the model output, as well as detailed discussions of the model equations, coefficients, and sensitivity analyses, are presented elsewhere (Scavia 1979). Only the results of the analysis of phytoplankton production and phosphorus cycling are considered here. To ensure that the results that follow are not biased by a 1-yr simulation or impacted seriously by initial conditions, I ran the model to approach dynamic equilibrium (20–25 yr). I found the relative influence of the various controls of phytoplankton dynamics and phosphorus cycling at equilibrium to be not unlike those described here.

The data used in this paper were obtained from STORET, the data management system of the U.S. Environmental Protection Agency, and are based on data collected by the Canada Centre for Inland Waters as part of the Lake Ontario Organic Particle Study (Stadelmann and Fraser 1974).

Results and Discussion

Although the model includes detailed process equations describing the food web and nutrient cycles in Lake Ontario, it is still a crude representation of reality. Therefore, before this model was used to assist in the examination of lake-scale phytoplankton production and phosphorus cycling, it was tested, in this context, as to its adequacy as a representation of the seasonal changes in relevant lakewide averaged properties in Lake Ontario. This was done by comparisons between measured and simulated properties. The comparisons are best for those properties measured with least uncertainty (i.e. chemical properties) and worst for those properties difficult to measure (i.e. phytoplankton and zooplankton carbon concentrations); however, these comparisons resulted in general agreement between measured and

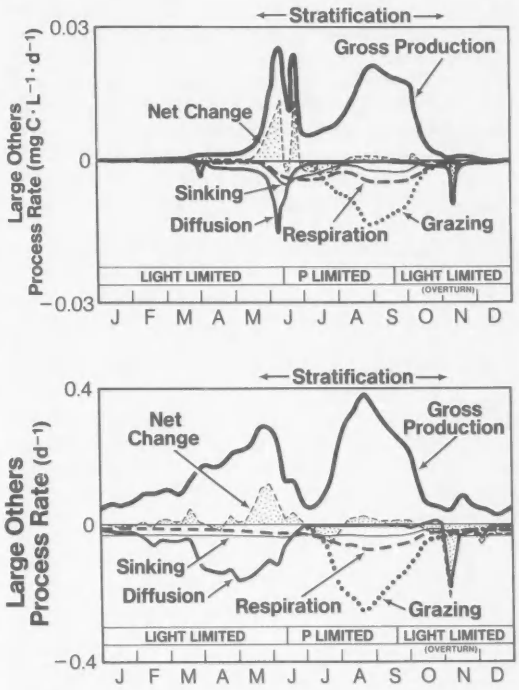

FIG. 3. Rate plots indicating simulated controls of epilimnion phytoplankton dynamics in Lake Ontario during 1972. Large Others represent nondiatoms greater than 20 μm. *Top* — Rates as d^{-1}, *bottom* — rates as mg $C \cdot L^{-1} \cdot d^{-1}$. Strippled area represents net growth rate.

simulated properties (Scavia 1979). These results demonstrate the model's ability to simulate the seasonal dynamics of phytoplankton, zooplankton, and the major nutrients; however, model output should also be compared to measurements of process rates to see how well the model simulates the internal dynamics of the system. Throughout the discussions that follow, both simulated and measured process rates are used to ensure that internal model dynamics are consistent with observations. I use process rates that were measured during IFYGL or measured in Lake Ontario during other years. Also, where certain processes were not observed in Lake Ontario, information from other field and laboratory studies are used for general comparisons. The simulated and measured rates were also in general agreement, which allows one to use the model to speculate about the relative importance of these processes.

Phytoplankton Dynamics

The simulated processes controlling the seasonal dynamics of the Large Others phytoplankton group in the epilimnion are shown in Fig. 3. The stippled area

on each graph indicates the net rate of change of the population biomass. In winter and early spring, this algal group, as well as the others, is controlled primarily by the balance between gross primary production and two physical processes, sinking and vertical mixing. During this time, phytoplankton gross production is limited mainly by the availability of light (Fig. 3), which is controlled both by the amount of incoming solar radiation and the depth to which the phytoplankters are mixed. Mixing between the two model layers becomes quite intense in early spring (Fig. 2), indicating that the mixing depth is the depth of the entire water column. This loss to the dark, deeper layers prevents substantial increases in algal biomass. Simons (1976) also found that even in three-dimensional simulations, the vertical mixing processes are very important in the early part of the year.

Riley (1942, 1946, 1963) and Riley et al. (1949) discussed the importance of the relationship between the depth of the euphotic zone and mixing depth. They suggested that phytoplankton can realize their productivity only after the thermocline begins to develop because it is only after this time that losses to the dark, non-productive strata are reduced. Significant increases in algal biomass occur only after midspring, when the surface waters in Lake Ontario begin to warm and the lake begins to stratify vertically (Fig. 3). At this time (early June), phytoplankton populations increase rapidly and the concentrations of the nutrients they assimilate begin to decrease. The concentrations of nutrients decrease because they also become relatively isolated from the nutrient-rich lower strata.

Diatoms, dominated by species (Table 1) with temperature optima in the lower range of Lake Ontario temperatures (Stoermer and Ladewski 1978), increase their productivity sooner than the other taxa. Consequently the diatoms assimilate available silicon and become limited by that nutrient before the other groups deplete the available phosphorus pool and become phosphorus-limited (Fig. 3). Late in the summer, phosphorus becomes limiting for diatoms as well. Phytoplankton production is limited by nutrients (silicon and phosphorus) from this time until the end of September, which has previously been suggested, based on mass balance considerations (Stadelmann and Fraser 1974) and on algal assays (Sridharan and Lee 1977).

During the same time period (late summer), grazing stress exerted by zooplankton becomes most intense (Fig. 3). This time history of simulated grazing pressure reflects the general seasonal pattern of crustacean zooplankton biomass (see Scavia 1979). All of the dominant species in Lake Ontario produced major biomass peaks during July or August in 1972 (McNaught et al. 1975). Also, for a previous year in Lake Ontario, Glooschenko et al. (1972) measured and compared the relative abundances of chlorophyll *a* and pheo-pigments and suggested that a high correlation between average percent pheo-pigment (relative to total chlorophyll *a*) and zooplankton abundance was probably a result of zooplankton grazing. On a lake-wide average basis, they found highest values for the percent of total chlorophyll *a* as pheo-pigments to occur in August–October. This further substantiates the simulation results in Fig. 3.

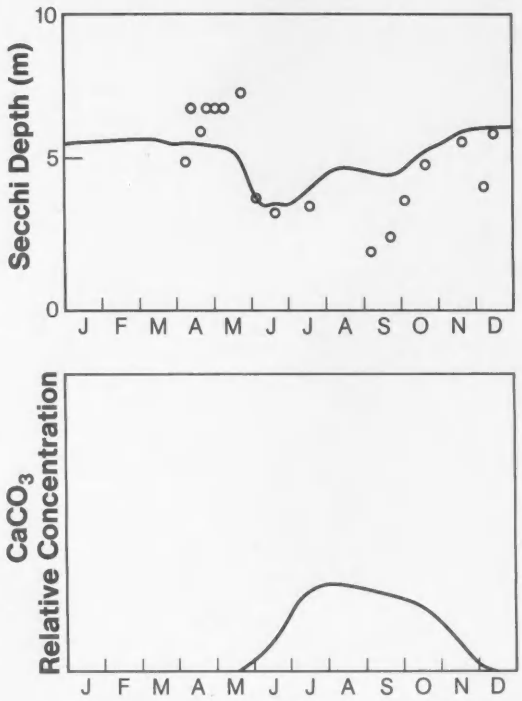

FIG. 4. *Top*: Simulated and observed Secchi depth. Simulation includes only effects of water color, algal carbon, and detrital carbon. *Bottom*: Simulated relative concentration of $CaCO_3$ precipitate forming in the epilimnion of Lake Ontario.

In late September the thermocline deepens (Fig. 2) and nutrient-rich, hypolimnetic water is mixed with epilimnetic water. Because of this increase in nutrient concentrations and the simultaneous increase in mixing depth, the algae again become limited by light. In early November the lake overturns and becomes vertically homogeneous and phytoplankton concentrations begin to approach winter values. Of course, this is a simplification of the three-dimensional effects discussed by Simons (1976); however in a one-dimensional model, all advective and dispersive processes are parameterized as vertical mixing.

Another factor in the control of phytoplankton production is the occurrence of "milky water" or $CaCO_3$ whitings in Lake Ontario. Kramer (1967) has demonstrated with equilibrium calculations that, for water temperatures greater than 13°C, Lake Ontario is saturated with respect to calcite. Eadie and Robertson

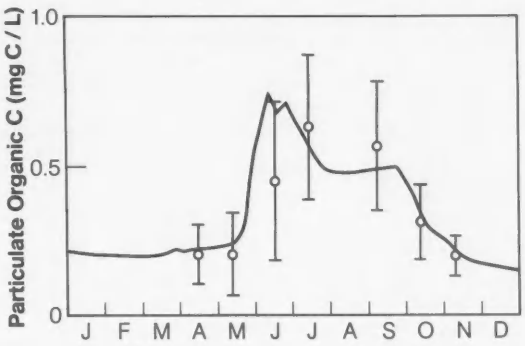

FIG. 5. Seasonal dynamics of data and model output for particulate organic carbon in the epilimnion (0–20 m) of Lake Ontario during 1972. Data are lakewide mean ± 1 SD.

(1976) discussed the seasonal trend in $CaCO_3$ saturation and indicated that in 1971–73 the epilimnion was highly supersaturated during the period of stratification. Satellite observations of these whitings (Strong and Eadie 1978) indicate that this phenomenon occurred in Lake Ontario during late August and September 1972. The whiting phenomenon has also been observed each year between 1972 and 1975, with the most intensive whitings observed in July–September 1973. Although the extent of the influence of these whitings on the lake's productivity is unclear at this time, Strong and Eadie suggest that the whitings may alter the light climate as well as scavenge nutrients, vitamins, and trace metals from the epilimnion.

Calculations of carbonate equilibrium, coupled with the ecological model, support the hypothesis that these whitings could have an impact on the lake's productivity and indicate that, if the whitings do affect primary production, they do so at a crucial time. Because the exact kinetics of crystal formation and dissolution are unknown and therefore not included in the model, the magnitude of the simulated concentration of $CaCO_3$ is not realistic; however, the timing of the peak of relative (or potential) concentration (Fig. 4) is consistent with theoretical considerations (Eadie and Robertson 1976) and observations (Strong and Eadie 1978). The potential impact of these whitings can be seen by examining seasonal variations in Secchi depth and concentration of particulate organic carbon (POC). Prior to and after the whitings, Secchi depths are accurately simulated by the model (Fig. 4). During these times, light extinction is due primarily to water color and particulate organic matter, both of which are accounted for in the Secchi depth calculations. The model, however, fails to simulate a 1.5-m decrease in Secchi depth in August. The concentration of POC (Fig. 5) during this time period also does not indicate that there should be a large change in transparency due to particulate organics. Simulation of the carbonate system (Fig. 4) indicates that the decreased transparency may well be caused by formation of $CaCO_3$ crystals during this time period. Also, during the period of maximum potential crystal formation (August–September), the phytoplankton is already shifting from being nutrient-limited to being light-limited (Fig. 3), and thus the timing of the crystal formation may also be critical to production.

PHOSPHORUS CYCLING

Although it is clear from the above analysis that nutrient limitation does not solely control phytoplankton dynamics, the role of nutrients, especially phosphorus and silicon, is certainly critical during the period of stratification. The phytoplankters dominant during the summer months in Lake Ontario are limited primarily by phosphorus, as demonstrated in the simulations discussed above and as shown by recent experimental work (Sridharan and Lee 1977). Therefore, to understand better the control of phytoplankton dynamics in Lake Ontario, one must investigate the processes influencing the cycling of phosphorus.

Figure 6 illustrates the seasonal changes in the simulated concentration of available phosphorus and rate of gross primary production in the epilimnion. As discussed above, after spring the phytoplankton become limited by nutrients and thus the production rate decreases sharply. It is interesting to note that, although the production rate has decreased considerably, it does not approach low winter values and, in fact, after the initial drop, the rate increases gradually. This sustained production proceeds at the same time that available phosphorus concentrations, both actual and simulated (Fig. 6, 8a), are extremely low. One might expect that, with phosphorus concentrations this low (<1 μg P/L) and sustained phytoplankton production, phosphorus assimilation by algae would rapidly drive the concentration of phosphorus to virtually zero and thus severely limit further phytoplankton productivity. However, this is not the case. It appears that the supplies of phosphorus during this time period are sufficient to balance phytoplankton assimilation. Thus the importance of internal cycling of phosphorus (Golterman 1973; Rigler 1973) cannot be ignored. The sum of the rates of detritus remineralization, phytoplankton and zooplankton excretion, and diffusion input from the hypolimnion, is approximately equal to the rate of assimilation by phytoplankton. This analysis (Fig. 6) suggests that decomposer input is about one-fourth of the excretion input from algae and zooplankton during summer stratification and that phosphorus input from the lower strata is important only before and after stratification.

To examine more closely the relationships among the various processes in this conceptual phosphorus cycle, I constructed a phosphorus flow diagram (Fig. 7) from model output averaged over the period of July–September and compared the results to available information. The sizes of the five phosphorus compart-

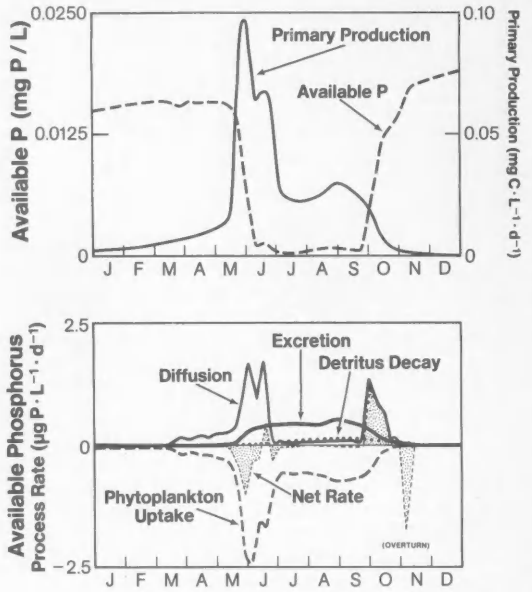

FIG. 6. *Top*: Simulated concentration of available phosphorus and rate of gross primary production in the epilimnion. *Bottom*: Rate plots indicating control of available phosphorus dynamics. Stippled area represents net rate of change.

ments are representative of Lake Ontario for this time period (Scavia 1979). The rate of conversion of available phosphorus to particulate phosphorus (Stadelmann and Fraser 1974) and the fluxes of phosphorus across the thermocline (Stadelmann and Fraser 1974; Burns and Pashley 1974) are also representative. Zooplankton grazing rates represent 50 and 53% of the animals' body phosphorus per day for omnivores and herbivores, respectively. This is consistent with general dry weight rations for small crustacean zooplankton (Parsons and Takahaski 1973). Zooplankton excretion rates represent 14 and 11% of body phosphorus per day for omnivores and herbivores, respectively. These rates agree with excretion rates summarized by Ganf and Blažka (1974) if one assumes for these animals a nitrogen to phosphorus body weight ratio of approximately 11 (cf. Parsons and Takahaski 1973). Quantitative information on other processes is not available and therefore, in those cases, model information alone will be used.

Figure 7 shows that it would take less than 1 d in the summer epilimnion for the phytoplankton to deplete the available phosphorus pool if there were no recycling and that external sources and hypolimnetic sources alone could not meet this algal phosphorus demand. In fact, this analysis indicates that 86% of the assimilated phosphorus is recycled within the epilimnion. Stadelmann and Fraser (1974) estimate this recycling to be

TABLE 2. Lake-wide average relative recycle rates of phosphorus in the epilimnion of Lake Ontario during stratification (Total rate = 0.629 μg P·L^{-1}·d^{-1}).

	Percent of total recycle rate
Phytoplankton excretion	32.8
Zooplankton excretion	
Herbivores	27.3
Omnivores	10.2
Total	37.5
Detritus decay	17.0
External load	3.2
Hypolimnion load	9.5

87–93% for the upper 20 m at a single station during the same time period.

Rigler (1973) suggested three possible sources for biological recycling of soluble PO_4-P: 1) direct release by ultraplankton (bacteria plus phytoplankton <30 μm), 2) excretion by zooplankton, and 3) enzymatic hydrolysis of organic compounds. Each of these sources has been studied separately and each has been cited as being very important. For example, Peters and Lean (1973), Peters and Rigler (1973), Ganf and Blažka (1974), Ferrante (1976), and others have examined the role of zooplankton excretion in the phosphorus cycle. Kuenzler (1970), Lean (1973a, b), Lean and Rigler (1974), and Lean and Nalewajko (1976) have demonstrated extracellular release of phosphorus by phytoplankton. The role of decomposers in nutrient cycling has been more difficult to quantify; however, in one study (DePinto and Verhoff 1977), incubation of laboratory phytoplankton cultures, when innoculated with a natural bacterial population from a eutrophic Michigan lake, resulted in remineralization of 74% of the algal phosphorus and nitrogen in 20–45 d. Golterman (1973) indicates that rates of autolysis may also be very high at times.

Rigler (1973) suggested that excretion by zooplankton and direct release from ultraplankton were equally important. The results of the present analysis (Fig. 7, Table 2) suggest that, for this five-compartment conceptualization, zooplankton excretion and direct release by phytoplankton are approximately equal and are the most important processes supplying phosphorus to the available pool. The rate of remineralization of detrital phosphorus is somewhat slower. This rate represents regeneration of approximately 1.7% of the detrital phosphorus per day, which is within the range measured by DePinto and Verhoff (1977). The general results (Fig. 7) are quite similar to Rigler's "provisional" model (his Fig. 5) when the different conceptualizations are considered and the relative phosphorus flows are compared.

The role of zooplankton in the phosphorus cycle must be emphasized. While the zooplankton has an obvious role in applying pressure to reduce algal con-

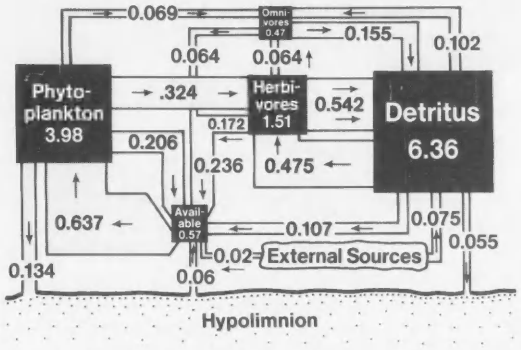

FIG. 7. Phosphorus flow diagram. Concentration in boxes are in $\mu g\ P/L$ and in pipes in $\mu g\ P \cdot L^{-1} \cdot d^{-1}$. These values are averaged over the period of July–September for the top 15 m. Phytoplankton, zooplankton, and detritus are evaluated in the model in terms of carbon and are converted to phosphorus for this figure by assuming a $C_{106}P_1$ atomic ratio.

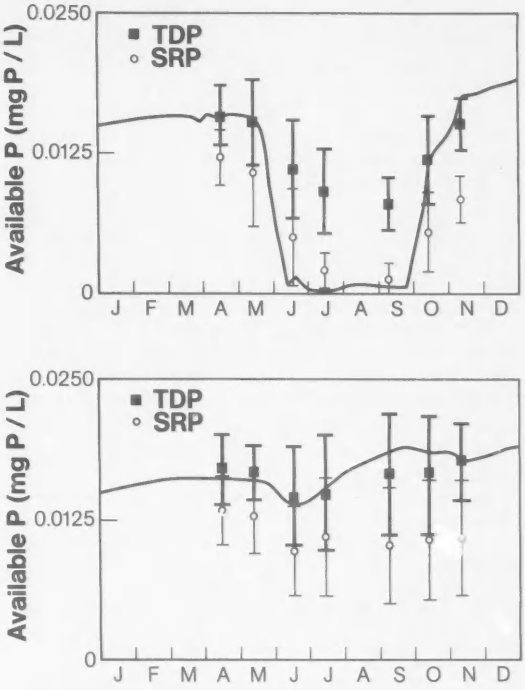

FIG. 8. Seasonal dynamics of TDP, SRP, and modeled available phosphorus (solid line) in the (a) epilimnion and (b) hypolimnion of Lake Ontario for 1972. Data are lake-wide mean ± 1 SD.

centrations (Fig. 3), it also appears to play a dual role in recycling phosphorus. Not only does the zooplankton input directly to the available nutrient pools through excretion, but it also serves as a supplier of detrital material (feces), which undergoes additional degradation by the decomposers and eventually adds to the available nutrient supply. Thus, it may well be that the zooplankton is principally responsible for the high recycling rates estimated by Stadelmann and Fraser in Lake Ontario.

PHOSPHORUS AVAILABILITY

The final aspect of the control of phytoplankton dynamics and phosphorus cycling to be considered here is phosphorus availability. It is commonly held that free orthophosphate (PO_4) is the phosphorus form most readily used by algae (Rigler 1973); however, assimilation of dissolved organic phosphorus (DOP), as demonstrated by Kuenzler (1970) and Paerl and Downes (1978), may also be important. Kuenzler showed reassimilation of DOP, through hydrolysis by extracellular alkaline phosphatase, by the same four marine algal species that had previously eliminated the DOP. Paerl and Downes demonstrated stimulation of P-starved *chlorella* when high molecular weight reactive P was added to the cultures. In addition to the direct use of DOP by algae, extracellular regeneration of PO_4 by other means also increases the potential supply of available phosphorus. Lean (1973a, b) demonstrated this regeneration of phosphorus in Heart Lake, and Peters (1978) estimated that available phosphorus in tributaries to Lake Memphremogog probably varied between 8 and 100% of total phosphorus. Cowen and Lee (1976) determined with algal assays that, of the phosphorus present in the tributaries to Lake Ontario, the fraction available for algal growth, on the average, consists of the soluble reactive phosphorus (SRP) pool plus approximately 20% of the sum of all other phosphorus forms. This type of "availability" is a function of the time scales of interest. If one were only interested in phosphorus available at one particular instant, PO_4 (if it could be measured) would probably be the best estimate; however, the amount of phosphorus eventually available over a given time period is a function of the length of the time period (Chamberlain and Shapiro 1973) and the strength of the hydrolytic potential present during that period.

If one assumes that, under the time scale of interest in this work, particulate phosphorus is unavailable, then the concentration of available phosphorus lies somewhere between total dissolved phosphorus (TDP) and PO_4. Figure 8a illustrates the seasonal changes of epilimnetic concentrations of TDP, available phosphorus (by definition in the model), and SRP (which is likely to be an overestimate of PO_4, Rigler [1973], Paerl and Downes [1978]). Modeled phosphorus is separated simply into forms that are available, unavailable, and in living plankton. The results shown here suggest that a large fraction of soluble unreactive phosphorus (SUP = TDP − SRP) is available to the phytoplankton in early spring and after fall overturn. Paerl

and Downes (1978) found, in a study of two New Zealand lakes, that higher molecular weight phosphorus was available for algal growth during 4-d incubations. It also appears from these simulations that most of the SUP, and in fact some of the SRP, is unavailable for algal growth during the period of summer stratification. These results imply that not only does the concentration of available phosphorus change seasonally (hence, phosphorus limitation), but the percent of TDP that is available also changes. This then implies that the composition of SUP, and thus the capacity to hydrolyze PO_4 from the SUP pool, changes seasonally.

In a study on Lake Kinneret, Berman (1970) found the extent of hydrolysis of organic phosphorus by natural phosphatases also changed seasonally. Hydrolysis in August, September, and November was lower than at other times; however, phosphatase activity in the lake was not severely reduced and, when phosphorus-laden water from a commercial fish pond was added to these samples, PO_4 was effectively released. He concluded that the lower rates of hydrolysis during August, September, and November were due to the existence of phosphorus forms resistant to enzymatic breakdown rather than due to a lack of enzymes in the lake. This may also be an explanation for the apparently large fraction of unavailable SUP in Lake Ontario during summer (Fig. 8a). The SUP pool may be composed of refractory compounds that are the end products of organic matter cycled several times through the food web. This suggestion of high rates of cycling was also evident from the analysis above (Fig. 7).

Berman also suggested that, under suitable conditions, gradual release of PO_4 from these more resistant phosphorus forms could occur. This may be occurring in the hypolimnion of Lake Ontario, where decomposition of unavailable phosphorus in the absence of primary production results in almost all of the dissolved phosphorus becoming available (Fig. 8b). This results in an increase in the availability of SUP in the epilimnion after fall overturn.

Summary

In other simulations of Lake Ontario phytoplankton, Thomann et al. (1975) and Scavia et al. (1976a) examined the relationship among modeled processes affecting phytoplankton and phosphorus dynamics. In the present study, an ecological model, based on process constructs posed by experimentalists over the past several years, driven by forcing functions that were measured and simulated specifically during IFYGL, and calibrated to lakewide averaged measurements of biological and chemical properties (Scavia 1979) was used in conjunction with in situ measurements and results from laboratory process studies to suggest important aspects of the control of phytoplankton production and phosphorus cycling in Lake Ontario during IFYGL.

The analysis indicated that, during 1972, Lake Ontario phytoplankton were controlled by several interacting processes. They were limited in spring by deep vertical mixing, in early summer by low phosphorus concentration, and in late summer by zooplankton grazing. Rapid internal recycle rates, especially due to zooplankton dynamics during late summer, provide phosphorus sufficient to maintain moderate phytoplankton production while SRP concentrations were generally low (1-2 μg P/L). Internal recycling rates of phosphorus were much higher than rates of external loading and influx from the hypolimnion. These controls of recycling and phytoplankton dynamics, estimated for IFYGL, are similar to controls suggested in the previous studies, which were based primarily on simulations.

It was also suggested, in the present study, that composition of the available phosphorus pool changes seasonally. It appears that the concentration of available phosphorus is most closely related to the total dissolved phosphorus pool in early spring and fall and is less than the concentration of the SRP pool during summer. The large fraction of soluble unreactive phosphorus that is unavailable to phytoplankton during summer may be composed of compounds relatively resistant to natural hydrolysis.

I have used an ecological model of Lake Ontario to synthesize information collected on individual ecosystem components and processes during IFYGL. Coupling this synthesis tool with traditional limnological analyses offered a more complete description of the control of phytoplankton dynamics and phosphorus cycling in Lake Ontario.

Acknowledgments

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Temperature and Photoperiod Effects on Gill (Na + K)–ATPase Activity in Chinook Salmon (*Oncorhynchus tshawytscha*)

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EWING, R. D., S. L. JOHNSON, H. J. PRIBBLE, AND J. A. LICHATOWICH. 1979. Temperature and photoperiod effects on gill (Na + K)–ATPase activity in chinook salmon (*Oncorhynchus tshawytscha*). J. Fish. Res. Board Can. 36: 1347–1353.

Juvenile Rogue River spring chinook salmon (*Oncorhynchus tshawytscha*) reared at two temperatures and photoperiods for a 2-yr period were examined for cyclic changes in gill (Na + K)–ATPase specific activity. Three peaks in activity were observed: a peak in October of the first year, a second higher peak the following May, and a peak in October of the second year. Exposure to photoperiods artificially advanced 3 mo (July–October) suppressed the October peak during the first year, but did not greatly alter the subsequent two peaks in activity. The temperature at which the fish were reared did not directly affect the changes in enzyme activity. However, growth rates varied in response to water temperature. Altered growth rates resulted in differences in the cyclic changes in gill (Na + K)-activated ATPase activity in the two temperature groups. A threshold of 8 cm was suggested as the minimal size at which the fish could respond to the appropriate photoperiod by altering the gill (Na + K)–ATPase activity.

Key words: (Na + K)–ATPase, chinook salmon, temperature, photoperiod, *Oncorhynchus tshawytscha*.

EWING, R. D., S. L. JOHNSON, H. J. PRIBBLE, AND J. A. LICHATOWICH. 1979. Temperature and photoperiod effects on gill (Na + K)–ATPase activity in chinook salmon (*Oncorhynchus tshawytscha*). J. Fish. Res. Board Can. 36: 1347–1353.

On a étudié les changements cycliques de l'activité spécifique de la (Na + K)–ATPase dans les branchies de jeunes saumons chinook (*Oncorhynchus tshawytscha*) de printemps de la rivière Rogue, maintenus à deux températures et photopériodes durant une période de 2 ans. On observe trois pics d'activité: un en octobre de la première année, un deuxième, plus élevé, en mai de l'année suivante et un troisième en octobre de la deuxième année. L'exposition à des photopériodes artificiellement avancées de 3 mo (juillet–octobre) élimine le pic d'octobre de la première année, mais n'affecte pas beaucoup les deux pics suivants. La température d'élevage des poissons n'affecte pas directement les changements d'activité enzymique. Cependant, le rythme de croissance varie selon la température de l'eau. Des rythmes de croissance modifiés produisent des différences de changements cycliques de l'activité de l'ATPase activée au (Na + K) des branchies chez les deux groupes de température. Nous suggérons un seuil de 8 cm comme taille minimale à laquelle les poissons peuvent réagir à la photopériode appropriée en altérant l'activité de la (Na + K)–ATPase dans les branchies.

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PARR–SMOLT transformation in anadromous juvenile salmonids includes changes which pre-adapt the fish to a life in the sea (Hoar 1976). These changes include an increase in guanine deposition (Johnston and Eales 1967, 1968), changes in lipid composition (Ota and Yamada 1974), alterations in ionic constituents (Houston and Threadgold 1963), and increased levels of gill (Na + K)–ATPase activity (Zaugg and McLain 1972; Zaugg and Wagner 1973; Giles and Vanstone 1976). At or near the time of parr–smolt transformation, downstream migration to the sea begins.

The interrelationship of these various aspects of parr–smolt transformation is not well understood. In particular, the relationship between the downstream migration and the physiological changes related to seawater survival is still unclear. A correlation between downstream migration and increased levels of gill (Na + K)–ATPase has been reported for steelhead trout (*Salmo gairdneri*) (Zaugg and Wagner 1973) and coho salmon (*Oncorhynchus kisutch*) (Lorz and McPherson 1976). These studies were conducted in coastal stocks with

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yearling fish during spring out-migrations. In chinook salmon (*Oncorhynchus tshawytscha*), however, migration may occur at diverse times during the year and at different ages (Reimers 1973; Lichatowich 1977; Krcma and Raleigh 1970).

In order to determine if such diverse patterns of outmigration in chinook salmon juveniles might be correlated with physiological changes, we followed the activity of gill (Na + K)–ATPase in Rogue River spring chinook over a 2-yr period in the laboratory and examined the influence of temperature and photoperiod on these changes. The results were compared with the characteristics of wild juvenile chinook in the Rogue River at time of outmigration as determined by extensive field studies (Lichatowich 1977) and by scale analysis of returning adults (Schluchter and Lichatowich 1977).

Materials and Methods

Fertilized green eggs of spring chinook salmon (1974 brood) from the Rogue River, Oregon, were transported on October 17, 1974, to the Oregon Department of Fish and Wildlife Research Laboratory at Corvallis, disinfected with an active iodine solution (Wescodyne), and incubated at 10°C in Health incubators until the yolk sacs were absorbed. The fry were then transferred on January 3, 1975, into circular, 1150-L tanks fitted with plywood hoods and supplied with running well water with an exchange rate of 3 L/min. Rearing temperatures were maintained at 8 ± 1°C or 12 ± 1°C for all tanks.

Lighting in the hooded tanks was provided by four 40-W fluorescent bulbs controlled by astral timers. Photoperiod lengths were adjusted on February 2, 1975 to normal seasonal day-lengths (±10 min) for Medford, Oregon as determined by the U.S. Naval Observatory. At the same time, photoperiods in selected tanks were artificially advanced by 3 mo. Thus, in July, fish under these advanced photoperiods were exposed to October day-lengths. Two 15-W incandescent bulbs regulated by astral timers were utilized to provide two 30-min twilight conditions daily in each tank. During the first year of rearing, each of the four environmental conditions (8 and 12°C under normal and advanced photoperiods) was duplicated in a second tank. Because differences between duplicate tanks were not significant, duplicate tanks were discontinued during the second year. All fish were fed to repletion daily on Oregon Moist Pellet in graded sizes.

Analyses of gill (Na + K)–ATPase activities were begun in May of the first year after the fish reached 5 cm in length. A random sample of 30 fish was collected from each experimental tank at monthly intervals during the first year and at 6-wk intervals during the second year. Each fish was stunned by a blow to the head, measured, weighed, and decapitated with a razor blade. The first gill arches were removed and blotted with Kimwipes. Gill filaments were dissected from the arches, weighed on a Cahn microgram electro-balance, and homogenized at 12.5 mg gill wet weight/mL of a homogenizing medium containing 0.2 mol/L sucrose, 0.02 mol/L Na_2EDTA, 0.01 mol/L 2-mercaptoethanol and 0.1 mol/L imidazole buffer, pH 7.2. (Na + K)–ATPase activity was determined by the whole homogenate method of Johnson et al. (1977). Protein was measured by a modification of the method of Lowry et al. (1955). All statistical comparisons were made at the 95% confidence level, using the Student's *t*-test.

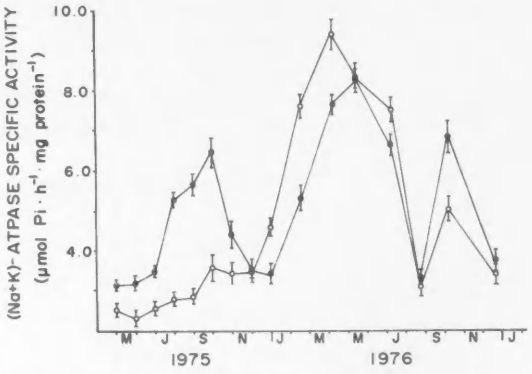

FIG. 1. Cyclic changes in gill (Na + K)–ATPase specific activity in juvenile chinook salmon reared at 8°C under either normal (-●-) or advanced (-○-) photoperiods. Points are means ± SE of random samples of 30 fish.

Results

Changes in Gill (Na + K)–ATPase Activity with Time and Photoperiod

Under normal lighting specific activity of gill (Na + K)–ATPase rose to a peak in October of the first year, then declined to a minimum in January (Fig. 1). During the second year, two additional peaks in gill (Na + K)–ATPase were observed, one in May and one in October. A group of spring chinook held at Cole Rivers Hatchery (Trail, Oregon) for a third year showed no further peaks in specific activity. Thus, three major peaks were seen during the first 2 yr of life, each separated by 6-mo intervals.

Changes in specific activity reflect changes in (Na + K)–ATPase activity rather than changes in protein. Protein concentrations (mg protein/mg wet weight gill tissues) remained constant throughout the study.

Photoperiod exerted a considerable influence on the cyclic changes in gill (Na + K)–ATPase activity during the first year. Under normal photoperiod at 8°C, (Na + K)–ATPase activity reached a peak in October of the first year, while in fish under an advanced photoperiod there was only a slight upward trend in specific activity. In juveniles reared at 12°C under normal photoperiod the October peak was less sharply defined than that in fish reared at 8°C. Under advanced photoperiods, there was an increase in specific activity until November. In December, specific activity decreased slightly, but the specific activity at this time was not significantly different ($P = 0.05$) from either the November or January activities.

During the second year, changes in gill (Na + K)–ATPase activity appeared less dependent upon photo-

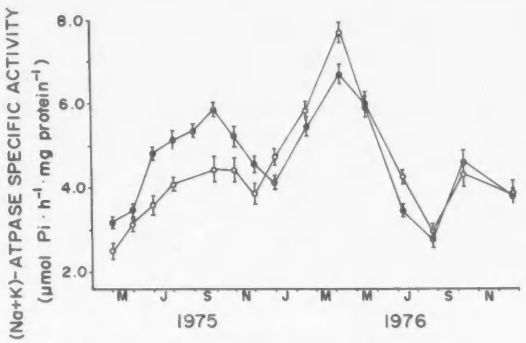

FIG. 2. Cyclic changes in gill (Na + K)–ATPase specific activity in juvenile chinook salmon reared at 12°C under either normal (-●-) or advanced(-○-) photoperiods. Points are means ± SE of random samples of 30 fish.

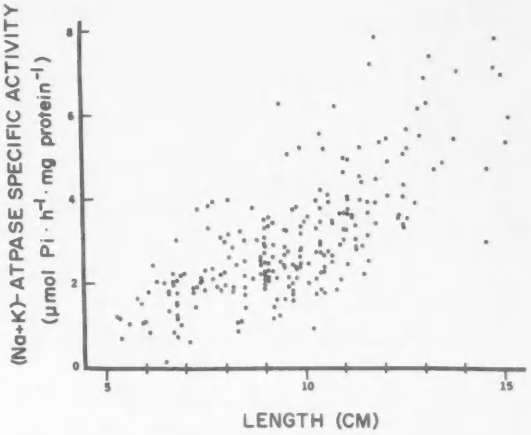

FIG. 3. Changes in gill (Na + K)–ATPase specific activity with fork length of juvenile chinook salmon. Points are derived from fish reared at 8°C under advanced photoperiods from May to December, 1975.

period. Under normal photoperiods, peaks in activity were seen in May and October. Under advanced photoperiods, the peaks in enzyme activity also occurred in May and October at 12°C and in April and October at 8°C (Fig. 1 and 2), although at this time, these fish were exposed to photoperiods corresponding to August and January. Thus, although fish at 8°C under an advanced photoperiod showed a peak in activity in April instead of May, this peak in activity occurred under a July photoperiod rather than a May photoperiod. At 12°C there was no significant difference ($P = 0.05$) between specific activities of the fish exposed to advanced and normal photoperiods during the second year, except at peak activity in May.

Fish Size and Gill (Na + K)–ATPase Activity

Fork lengths of fish and specific activities of gill (Na + K)–ATPase were correlated throughout the first year in all experimental conditions. Larger fish usually had greater gill (Na + K)–ATPase activity than smaller ones, regardless of the time of year. This relationship is demonstrated in Fig. 3 in which specific activities for juveniles reared under advanced photoperiods from May until December 1975 are plotted against fork length. Under those conditions, photoperiod-induced cyclic changes in gill (Na + K)–ATPase were blocked. The relationship in Fig. 3 can be described by the regression equation: Fork length = 6.48 + 1.043 (specific activity), $r^2 = 0.530$ ($n = 237$). During the second year, this relationship was no longer valid.

Regression of weight on specific activity gave a similar correlation ($r^2 = 0.565$). This relationship was not used in the following analyses, however, because weight can be a much more variable measurement than fork length due to variations resulting from the timing of previous feeding, efficiency of blotting for weight measurements and changes in lipid content throughout the year (Fessler and Wagner 1969).

Gill (Na + K)–ATPase activity in fish reared under normal photoperiods at 8°C was examined by size class in September and October of the first year. These activities were compared with levels of gill (Na + K)–ATPase activity calculated by using the equation above relating fork length and specific activity. Differences between calculated activities and observed activities could be ascribed to the influence of photoperiod on the level of (Na + K)–ATPase activity in the gill. Although the sample size in most cases was small, a relationship between the size of the fish and the extent of its response to photoperiod was found (Fig. 4). Gill (Na + K)–ATPase activities in small fish deviated little from the values expected from the fork length–specific activity relationship. In the range of 10–12 cm, the increase in gill (Na + K)–ATPase activity in response to September and October photoperiods reached a maximum. As the fish exceeded 12 cm, the difference between expected and observed values again declined. These results suggest that maximal changes in gill (Na + K)–ATPase activity in response to photoperiod occur only within narrow size limits.

Fish Growth and Gill (Na + K)–ATPase Activity at Two Temperatures

Under repletion feeding, growth of fish (cm fork length/d) in all tanks was linear with time during the first year. Growth, however, varied with water temperature and photoperiod (Tables 1 and 2). Growth rates were greatest at 12°C under normal photoperiods and least at 8°C under advanced photoperiods. Growth under advanced photoperiods was somewhat slower than under normal photoperiods (Tables 1 and 2).

Water temperatures at which the fish were reared did not seem to have a major effect on gill (Na + K)–

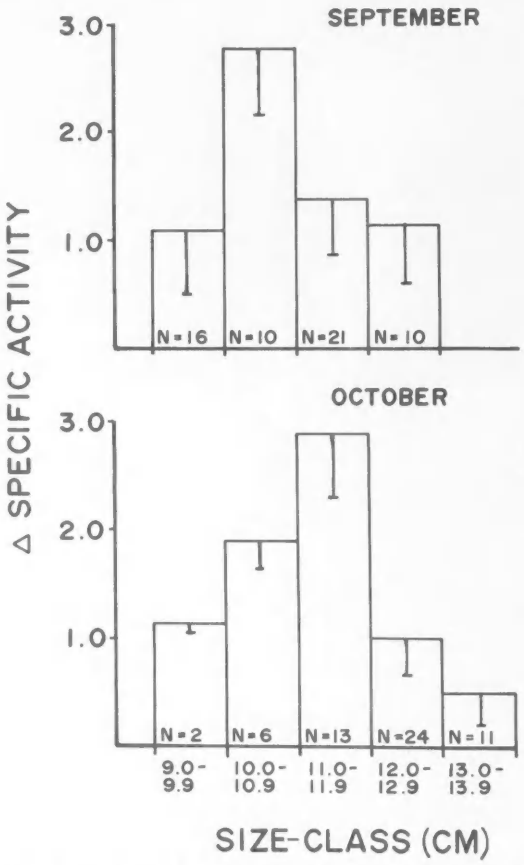

FIG. 4. Cyclic changes in gill (Na + K)–ATPase specific activity in relation to fork length of juvenile chinook salmon in September and October. Fish were reared at 8°C under normal photoperiods. Δ specific activity is the average (±SE) difference between observed specific activities and those calculated from the regression equation derived from Fig. 3.

ATPase activity. When the relationship between enzyme activity and fork length described above was used to correct for changes in activity due to differences in growth rate between 8 and 12°C, there was no significant difference ($P = 0.05$) in enzyme activity between fish reared at 8°C and those reared at 12°C. When rates of increase of (Na + K)–ATPase activity were compared between 8 and 12°C under advanced photoperiods (to prevent cyclic changes in response to photoperiod), the activity increased at a rate related to the growth rate of the fish (Table 3). There was an increase in activity of approximately 0.26 μmol $Pi \cdot h^{-1} \cdot mg\ protein^{-1}$ for every centimetre change in fork length of the fish, regardless of the temperature at which the fish was reared. Under normal photoperiods, the maximum specific activity attained at the October peak was not significantly different at the 95% confidence level for fish reared at 8 and 12°C. During the second year, maximal levels attained during April and October peaks were significantly lower ($P = 0.05$) at 12°C.

Discussion

In his recent review on parr–smolt transformation, Hoar (1976) associated parr–smolt transformation with downstream migration of the juveniles. In two species of salmonids, the timing of downstream migration and increases in gill (Na + K)–ATPase are highly correlated. Zaugg and Wagner (1973) found that high levels of gill microsomal (Na + K)–ATPase were correlated with the downstream migration of juvenile steelhead. Kerstetter and Keeler (1976) confirmed this in hatchery-reared steelhead on the Trinity River. W. Zaugg and H. Lorz (Aquaculture Field Station, Cook, WA, unpublished data) and Lorz and McPherson (1976) found similar correlations in coho salmon.

Present data indicate that there are at least three peaks in gill (Na + K)–ATPase activity in Rogue River spring chinook salmon. The peak in activity in October occurs at a time when approximately 75–85% of the juveniles which later returns as adults first enter the ocean, as determined by analysis of scales of returning adults (Schluchter and Lichatowich 1977). At this time, maximum numbers of juveniles are captured near the mouth of the Rogue River (Lichatowich 1977). Coincident with the second peak in gill (Na + K)–ATPase activity in May, there is a small population of yearling chinook which migrate to the sea and subsequently comprise 10% of the adult population (Schluchter and Lichatowich 1977). These results from scale analyses are complemented by field observations of small peaks in yearling chinook captured in the Rogue River at this time (Lichatowich 1977). Thus, there is evidence for a correlation between migration, seawater entry, and elevated gill (Na + K)–ATPase levels in Rogue River spring chinook juveniles.

Photoperiod appears to strongly influence the appearance of the peak in specific activity in October of the first year. Under photoperiods advanced 3 mo, no distinct peak in activity was observed at this time. We suggest that this peak in activity did not occur because the fish which were subjected to September and October photoperiods in June and July were too small to respond to the photoperiod stimulus. During September and October, when these fish were subjected to photoperiods of December and January, there was also no peak in specific activity. Thus, cyclic changes in activity were suppressed during the first year by the advanced photoperiods.

During the second year, the influence of photoperiod on the cyclic changes in specific activity was greatly reduced. The peak in activity occurred 1 mo earlier in fish reared at 8°C under advanced photoperiod than in those

TABLE 1. Fork lengths (mean ± SE) of juvenile spring chinook salmon (*Oncorhynchus tshawytscha*) under two temperature and two photoperiod regimes. Sample sizes 30–60 fish.[a] (*$P < 0.05$ for advanced compared to normal photoperiod.)

Date	Normal photoperiod		Advanced photoperiod	
	8°C	12°C	8°C	12°C
June 1975	7.75±0.09	8.92±0.13	7.31±0.09	9.00±0.13
August 1975	9.99±0.13	12.11±0.08	9.17±0.18	11.77±0.22
October 1975	12.09±0.15	15.52±0.24	10.72±0.17*	14.57±0.31*
December 1975	12.94±0.27	17.49±0.25	11.70±0.17*	14.99±0.57*
Feb. 17, 1976	14.61±0.48	20.18±0.55	13.20±0.32*	17.04±0.57*
Apr. 7, 1976	16.88±0.38	22.45±0.43	15.27±0.38*	18.79±0.61*
July 6, 1976	18.11±0.37	24.57±0.25	17.39±0.30*	23.65±0.61*
Aug. 24, 1976	19.20±0.23	26.56±0.48	18.97±0.31	24.47±0.81*
Oct. 7, 1976	20.15±0.41	27.65±0.32	20.65±0.44	24.73±0.48*

[a]Values for 1975 are averages of two 30-fish samples from replicate tanks. Values for 1976 are averages of single 30-fish samples.

TABLE 2. Regression equations of length on calendar day for spring chinook juveniles during 1975. (Relationships were calculated from means of samples of 60 fish for eight monthly sampling periods during 1975. Slopes represent growth rates in centimetres per day.)

Temperature	Normal photoperiods	Advanced photoperiods
8°C	$0.0292X+3.531$, $r^2 = 0.99$	$0.0238X+3.981$, $r^2 = 0.97$
12°C	$0.0487X+1.662$, $r^2 = 0.99$	$0.0373X+3.402$, $r^2 = 0.95$

TABLE 3. Comparison of growth rates and rates of increase in (Na+K)–ATPase specific activity at two temperatures. (Relationships were calculated from means of 60 samples at eight monthly sampling periods during 1975. Slopes represent either growth rates in centimetres per day or changes in specific activity per day. All fish were held under advanced photoperiods to inhibit cyclic changes in specific activity resulting from photoperiod.)

Temperature (°C)	Regression equations	
	Length on calendar day	Specific activity on calendar day
8	$0.0238X+3.981$, $r^2 = 0.97$	$0.00641X+1.49$, $r^2 = 0.85$
12	$0.0373X+3.402$, $r^2 = 0.95$	$0.00934X+1.77$, $r^2 = 0.83$
Ratio of slopes	0.64	0.69

reared at 8°C under normal photoperiod. These results suggest that there was some influence of photoperiod on the cyclic changes in activity. However, the peak was delayed two months from that expected if the cyclic changes in activity were regulated solely by photoperiod. At 12°C, fish reared under both advanced and normal photoperiods had peaks in activity in May. All photoperiod and temperature regimes had similar peaks in activity during October of the second year. These results are consistent with the hypothesis that both photoperiod and endogenous rhythms regulate the cyclic changes in (Na + K)–ATPase activity in gills of juvenile chinook salmon and that the photoperiod influence is diminished during the second year of life. Interactions of exogenous and endogenous rhythms are well known in birds and mammals (Aschoff 1960; Gwinner 1977).

The influence of fish size on gill (Na + K)–ATPase activity has not been extensively examined in other studies. Zaugg and McLain (1972) reported that there was no relationship between size and gill (Na + K)–ATPase activity in juvenile chinook salmon. However, these studies were done on yearling spring chinook of unspecified size. In Rogue River spring chinook, we found that size is important in the regulation of gill (Na + K)–ATPase activity only during the first year of life. This period, however, is coincident with the timing of the major chinook juvenile outmigration in the Rogue River.

In a limited size range, the size of the juvenile chinook

appears to regulate the extent to which the gill (Na + K)–ATPase activity is increased in response to photoperiod. In the range of 10–12 cm, there was a large cyclic change in specific activity from September to November (Fig. 4). However, in both larger and smaller size-classes, there was little change in specific activity. It has been shown from the analysis of adult scales that approximately one-half of the returning spring chinook adults in the Rogue River entered the ocean as juveniles at a size of 10–12 cm (Schluchter and Lichatowich 1977).

These results suggest that there may be a minimum length which a juvenile must attain before cyclic changes in gill (Na + K)–ATPase specific activity occur. We hypothesize that the length which must be attained by September–October is greater than 8.0 cm. Under advanced photoperiods, juveniles which had attained a fork length of 7–8 cm (Table 1) did not show a peak in specific activity in July, even though the day-lengths were those of October. A minimum size for smolting has been reported for steelhead (Wagner et al. 1963), although the size threshold was approximately 16 cm long, almost twice the hypothesized critical size for spring chinook. Experiments are currently in progress to examine this size threshold.

The relationship between water temperature and gill (Na + K)–ATPase activity appears to be different for chinook from that reported by Zaugg and McLain (1976) for coho salmon and by Adams et al. (1973) for steelhead trout. Increased activity of gill (Na + K)–ATPase in fish reared at 12°C during the first year could be accounted for on the basis of increased growth rates at the higher temperature and the relationship between fork length and gill (Na + K)–ATPase activity (Fig. 3). During the second year, however, maximal values of gill (Na + K)–ATPase activity were lower at 12°C than at 8°C. The former temperature is somewhat below the critical temperature of 15°C reported for steelhead and coho (Zaugg et al. 1972; Zaugg and McLain 1976), but is close to the temperature of 13°C which was suggested to affect steelhead (Zaugg and Wagner 1973). Fish residing in the Rogue River might be expected to exhibit higher temperature tolerances through adaptation to high temperatures often present during the summer months.

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Body Size and Feeding Ecology as Alternatives to Taxonomy for the Study of Limnetic Zooplankton Community Structure

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Three different characterizations of limnetic zooplankton communities from lakes of the Bruce Peninsula, Ontario are compared with respect to their usefulness in limnological studies: a conventional taxonomic one, one based on the body size and feeding ecology of the organisms, and one based on image analysis of the particle size spectrum. In comparison with the taxonomic characterization, the ecological and image analysis characterizations reflect zooplankton community features which are more closely related to the efficiency and nature of energy flow through pelagic ecosystems. Furthermore, it is demonstrated that these features have stronger statistical relations to morphometric and hydrological properties of lakes. The ecological and image analyses are thus not only more useful in studies seeking to make predictions about lake function from lake type but actually require less time, money and expertise in the acquisition of zooplankton data. In comparisons of zooplankton communities from the disparate faunal regions of the Bruce Peninsula, Ontario and southeast Asia, particularly Sri Lanka, more useful functional insights emerge from a body size-feeding ecology characterization than from a taxonomically based one.

Key words: community structure, image analysis, Bruce Peninsula, tropical zooplankton, zoogeography, multivariate analysis

SPRULES, W. G., AND L. B. HOLTBY. 1979. Body size and feeding ecology as alternatives to taxonomy for the study of limnetic zooplankton community structure. J. Fish. Res. Board Can. 36: 1354–1363.

Trois caractérisations différentes de communautés zooplanctoniques limnétiques de lacs de la péninsule de Bruce en Ontario sont comparées quant à leur utilité dans des études limnologiques: une caractérisation taxonomique conventionnelle, une fondée sur la taille du corps et l'écologie alimentaire des organismes et une troisième fondée sur l'analyse de l'image du spectre de grosseurs des particules. Par comparaison avec la caractérisation taxonomique, les caractérisations écologiques et par analyse de l'image reflètent les propriétés de la communauté zooplanctonique plus étroitement liées à l'efficacité et à la nature du flux énergétique dans les écosystèmes pélagiques. En outre, nous démontrons que ces propriétés ont d'étroites relations statistiques avec les caractères morphométriques et hydrologiques des lacs. Les analyses écologiques et par l'image sont donc, non seulement plus utiles dans des études visant à prédire la fonction des lacs à partir des types de lacs, mais exigent en réalité moins de temps, d'argent et de compétence lorsqu'il s'agit de recueillir des données sur le zooplancton. En comparant des communautés zooplanctoniques de régions fauniques aussi différentes que la péninsule de Bruce en Ontario et le Sud-est asiatique, en particulier Sri Lanka, il est plus de concepts fonctionnels utiles qui émergent d'une caractérisation taille du corps-écologie alimentaire que d'une fondée sur la taxonomie.

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THE relations between limnological characteristics of lakes and the structure of their zooplankton communities have served as a basis for a) studying zoogeographic trends and ecological patterns in freshwater zooplankton (Anderson 1974; Carter 1971; Patalas 1975; Whittaker and Fairbanks 1958), b) for using zooplankton associations as indicators of water quality (Fuller et al. 1977; Stemberger and Gannon 1977), and c) for grouping lakes into "types" useful for experimental, modelling, and management purposes (Patalas 1971; Sprules 1977). Community structure is generally defined as some function of the abundances of the taxa composing the community but it is becoming increasingly apparent that energy flow within aquatic ecosystems is much more dependent on the body size and feeding ecology of organisms than it is on their taxonomic status (Kajak and Hillbricht-Ilkowska 1972; Kerr 1971, 1974a; Sheldon et al. 1977; Steele and Frost 1977). Thus, in addition to the established importance

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of body size in the susceptibility of zooplankton to predation and competition (Allan 1976; Hall et al. 1976; Lynch 1977; Neill 1975), the efficiency of energy flow from phytoplankton to zooplankton, the efficiency of zooplankton production, and planktivorous fish production can also be shown to depend on zooplankton body size (Galbraith 1975; Gliwicz 1969; Hillbricht-Ilkowska 1977; Kerr 1974b; Morgan et al. 1978; Northcote 1972; Petrova et al. 1975; Sprules 1979a). Hence, because they are correlates of important ecological processes in lakes, body size and feeding ecology could be more informative descriptors of community structure than is taxonomic composition. Our study was undertaken to show that such a characterization of zooplankton communities has stronger relations to limnological properties of lakes of the Bruce Peninsula, Ontario than does a taxonomic one, and that this more functional approach to zooplankton has advantages for the comparisons of zooplankton community structure in tropical and temperate lakes.

Materials and Methods

Forty-one lakes, some with multiple basins to give a total of 44 water bodies, on the Bruce Peninsula, Ontario were visited once each during July and August, 1975. The limnetic zooplankton community of each lake was sampled during daylight hours with a 30-cm conical townet of 80-μm nylon mesh drawn from bottom to surface at the point of maximum depth of each basin. Samples were preserved in an aqueous solution of 4% formalin and 4% sucrose (Haney and Hall 1973). Subsamples of 5–10% were analyzed at 25$\times$ under a dissecting microscope by recording presence or absence of zooplankton species in each of ten randomly selected fields. The relative abundance of a given species in a given lake could thus have an integer value from zero (species absent) to ten (species abundant, i.e. present in all fields counted). Species which did not appear in one of the ten fields examined but which had been observed in the initial survey of the sample were assigned a value of 0.1. Early copepodites which could not be identified, nauplii, and rotifers were not counted. Taxonomic keys by Brooks (1957), Brandlova et al. (1972), Czaika and Robertson (1968), and Edmondson (1959) were used for identification.

In addition to these taxonomic data, two other characterizations of the communities were determined. For the first, the ecological characterization, the 42 species were reorganized into eight size-feeding ecology categories (Table 1) on the basis of mean adult length determined from a cross section of the lakes and feeding type (herbivore or carnivore) determined from the literature and personal experience. For each lake the abundances of the species making up a particular category were then summed and expressed as a proportion of the maximum possible abundance for the category (simply the number of species in the category times 10, the maximum possible abundance per species). For the second, the image analysis characterization, particles in 42 of the original 44 lake samples were sized and counted with a Quantimet 720 image analyzer (Image Analyzing Computers Ltd., U.K.) (Fisher 1971). The samples were prepared by first staining small aliquots in a 2% solution of methylene blue for at least an hour. After excess stain was removed by washing in flowing water for 1 min, all particles (crustacean zooplankton, rotifers, detritus, net phytoplankton) were stained deep blue. These stained particles were washed into a shallow petri plate and photographed against diffused light on high contrast copy film at one-quarter life size. Four negatives from nonoverlapping fields, each typically containing 500–1200 particles, were made per sample. Photography of the particles is not an absolute requirement for the image analysis but the increases in specimen contrast obtained permit reliable counting of all particles resolved on the negative (L. B. Holtby, D. R. S. Lean, Canada Centre for Inland Waters and P. A. Peach, Brock University, unpublished data). The photographed particles were counted in 15 size categories corresponding to actual particle sizes of 0.05–2.00 mm, although for analysis the comparatively rare particles greater than 0.83 mm were grouped into one category. Because most of the particles in these samples were spherical or nearly so, sizing distortions arising from the oblique orientation of elongated particles to the machine scan lines is minimal. Counts were expressed as proportionate numerical abundances of particles in the various size-classes.

Dr Harold Harvey, University of Toronto provided a range of standard limnological data for these lakes including measures of lake size and depth, flushing rate, number of fish species and a variety of surface chemical determinations such as sulphate, total dissolved solids, hardness, and pH.

Zooplankton data from 29 Sri Lanka reservoirs greater than 300 ha in surface area, Bhopal Lake, India, and Bhumipol Reservoir, Thailand were kindly made available by C. H. Fernando, University of Waterloo. He also augmented published data from Lake Toba, Sumatra (Brehm 1933; Kiefer 1933), Lake Lanao, Philippines (Frey 1969; Lewis 1974), and Grand Lac, Cambodia (Mizuna and Mori 1970) (summarized in Fernando 1979). Except for two which were visited in 1965, the reservoirs in Sri Lanka were all sampled between 1969 and 1974 with conical townets in open water. Except for one sampled in July, all were sampled from December to March, the rough subtropical equivalent of the north temperate stable midsummer period. Although the samples were taken in open water, littoral species are included to a greater or lesser extent depending on rainfall pattern near sampling time (Fernando 1979). In total, presence–absence zooplankton data in 34 water bodies in Sri Lanka and adjacent southeast Asia were used in this analysis (Table 1).

Principal components analyses were used to summarize the major lake to lake variation in community structure of the Bruce lakes for each of the three characterizations of zooplankton. For the taxonomic data, 22 very rare species which generally occurred in fewer than 10% of the lakes were first removed from the raw data leaving a matrix of the relative abundance of 20 species in 44 lakes. Principal components were extracted from the species variance–covariance matrix. For the ecological characterization, the matrix of the proportionate occurrence of eight size-feeding ecology categories in 44 lakes were normalized by an arcsin square root transformation and components extracted from the categories variance–covariance matrix. Similarly for the image analysis data the proportionate numerical abundances of particles in eight size classes for 42 lakes were normalized by the same transformation and components extracted from the variance–covariance matrix of size categories. We related these patterns in zooplankton community structure to limnological characteristics of the Bruce lakes using a forward stepwise multiple regression

TABLE 1. Occurrence of species arranged according to their ecological classification for lakes in the region of the Bruce Peninsula and for southeast Asian lakes.

Bruce Peninsula		Southeast Asia	
Species	% of lakes	Species	% of lakes
Herbivore 1 (< 0.50 mm)		Herbivore 1 (< 0.50 mm)	
Alona guttata Sars	2.2	*Alona monocantha* Sars	2.9
Alonella nana (Baird)	2.2	*Biapertura intermedia* (Stingelin)	8.8
Bosmina longirostris (O. F. Müller)	98	*Biapertura karua* (King)	12
Chydorus sphaericus (O. F. Müller)	39	*Biapertura verrucosa* (Sars)	21
Pleuroxus procurvus Birge	6.8	*Bosmina longirostris* Müller	8.8
Herbivore 2 (0.51–0.80 mm)		*Bosminopsis dietersi* Richard	24
		Ceriodaphnia cornuta Sars	85
Acroperus harpae Baird	14	*Ceriodaphnia dubia* Richard	2.9
Alona rectangula Sars	2.2	*Ceriodaphnia quadrangula* (Müller)	5.9
Camptocercus rectirostris Schødler	4.5	*Chydorus barriosi* Richard	44
Ceriodaphnia lacustris Birge	75	*Chydorus eurynotus* Sars	18
Drepanothrix dentata (Eurén)	2.2	*Chydorus parvus* Daday	2.9
Graptoleberis testudinaria (Fischer)	2.2	*Chydorus sphaericus* Müller	2.9
Ilyocryptus spinifer Herrick	4.5		
Leydigia quadrangularis (Leydig)	2.2	Herbivore 2 (0.51–0.80 mm)	
Pleuroxus denticulatus Birge	2.2		
Scapholeberis aurita (Fischer)	2.2	*Alona harpularia* Sars	2.9
		Diaphanosoma excisum Sars	62
Herbivore 3 (0.81–1.20 mm)		*Diaphanosoma modigliani* Richard	5.9
		Diaphanosoma sarsi Richard	18
Alona affinis (Leydig)	2.2	*Dunhevedia crassa* King	2.9
Alona quadrangularis (O. F. Müller)	4.5	*Echinisca triserialis* (Brady)	15
Daphnia retrocurva Forbes	27	*Moina micrura* Kurz	76
Diaphanosoma brachyurum (Liéven)	75	*Pseudochydorus globosus* (Baird)	2.9
Diaptomus ashlandi March	2.2		
Diaptomus minutus Lilljeborg	23	Herbivore 3 (0.81–1.20 mm)	
Diaptomus oregonensis Lilljeborg	57		
Diaptomus reighardi Marsh	32	*Allodiaptomus raoi* Kiefer	2.9
		Chydorus ventricosus Daday	2.9
Herbivore 4 (> 1.2 mm)		*Daphnia lumholtzi* Sars	26
		Neodiaptomus blachei (Brehm)	2.9
Daphnia catawba Coker	4.5	*Leydigia australis* Sars	5.9
Daphnia dubia Herrick	4.5	*Neodiaptomus botulifer* Kiefer	2.9
Daphnia galeata Sars *mendotae* Birge	11		
		Herbivore 3 (0.81–1.20 mm)	
Herbivore 4 (> 1.2 mm)			
		Phyllodiaptomus annae Apstein	65
Holopedium gibberum Zaddach	11	*Thermocyclops crassus* (Fischer)	59
Sida crystallina (O. F. Müller)	27	*Tropodiaptomus doriae* (Richard)	2.9
Simocephalus serrulatus (Koch)	4.5	*Tropodiaptomus gigantoviger* Brehm	2.9
Carnivore 1 (< 0.90 mm)		Herbivore 4 (> 1.20 mm)	
Orthocyclops modestus (Herrick)	2.2	*Eudiaptomus cinctus* (Gurney)	2.9
Polyphemus pediculus (L)	18	*Heliodiaptomus viduus* (Gurney)	12
Tropocyclops prasinus mexicanus Kiefer	61	*Ilyocryptus spinifer* Herrick	2.9
		Leydigia acanthocercoides (Fischer)	2.9
Carnivore 2 (0.91–1.20 mm)		*Neodiaptomus schmackeri* (Poppe & Richard)	29
Cyclops bicuspidatus thomasi Forbes	14	*Phyllodiaptomus visnu* Daday	2.9
Cyclops vernalis Fischer	61	*Psuedosida bidentata* Herrick	15
Mesocyclops edax (Forbes)	82	*Simocephalus vetulus* Müller	8.8
Mesocyclops leukarti (Claus)	9.1	*Tropodiaptomus australis* Kiefer	24
		Tropodiaptomus vicinus Kiefer	2.9
Carnivore 3 (1.21–1.50 mm)			
		Carnivore 1 (< 0.90 mm)	

TABLE 1 (*Concluded*)

Bruce Peninsula		Southeast Asia	
Species	% of lakes	Species	% of lakes
Eucyclops agilis (Koch)	4.5		
Eucyclops speratus (Lilljeborg)	2.2	*Mesocyclops leukarti* (Claus)	100
Carnivore 4 (> 1.5 mm)			
Chaoborus Lichtenstein sp.	34		
Epischura lacustris Forbes	59		
Leptodora kindtii (Focke)	14		
Macrocyclops albidus (Jurine)	2.2		

procedure. The lake score on the principal components was the dependent variable and the limnological data (with the exception of pH, normalized by a natural logarithm transformation) were the independent variables.

To compare temperate and tropical lakes in terms of the size-feeding ecology structure of their zooplankton communities, we converted the taxonomic data from the Bruce lakes to species presence–absence to make them compatible with the southeast Asian lake data. The Asian lake species were reorganized into the same ecological categories as used for the Bruce lakes (Table 1) on the basis of size and feeding ecology information from C. H. Fernando and from published accounts (Edmondson 1959; Goulden 1968; Green 1967; Gurney 1933; Monakov 1976; Smirnov 1974, 1976; Zaret 1975). A new matrix of 78 lakes (44 Bruce plus 34 Asian) and eight size-feeding ecology categories was created by determining the proportion of the total species in each lake which belonged to a particular category. These proportions were normalized by an arcsin square root transformation and principal components extracted from the variance–covariance matrix of categories.

All of the statistical analyses were done at the University of Toronto computing center using the statistical programs NTSYS (Rohlf et al. 1971), SPSS (Nie et al. 1975), and GENSTAT (Alvey et al. 1977).

Results and Discussion

Species correlations with the first component (Table 2) indicate that the most important variation in the taxonomic structure of zooplankton communities is from those with relatively high abundances of *B. longirostris* and *Diaptomus reighardi* to those with relatively high abundances of *Diaphanosoma brachyurum*, *Diaptomus minutus*, *Daphnia retrocurva*, *E. lacustris*, *Diaptomus oregonensis*, and *C. b. thomasi*. A secondary variation is from lakes in which *Diaptomus reighardi* and *D. minutus* predominate to those in which *D. oregonensis*, *C. vernalis*, and *T. p. mexicanus* are relatively abundant while the third component contrasts lakes dominated by *C. vernalis*, *D. minutus*, *B. longirostris*, and *Ceriodaphnia lacustris* with those in which *D. oregonensis* and *D. reighardi* predominate. While these patterns in taxonomic structure of communities are of interest in zoogeographic and autecologic studies, they do not directly reflect trophodynamic processes. We suggest it is of greater relevance to studies of energy flow through a lake to know, for instance, that a large herbivorous organism (which grazes on certain particles and is utilized by particular fish) is present, not whether it is *Daphnia catawba* or *Holopedium gibberum*. The taxonomic designation of a zooplankter subsumes much ecological information such as size, feeding ecology, growth rate, color, life cycle, etc. But since body size and feeding ecology seem to most closely reflect the ecological role of a species then these parameters alone should be used as a basis for characterizing communities.

In this respect patterns reflected in the ecological classification provide a better basis for understanding important functional differences among lakes than do the taxonomic ones. Correlations with the first ecological component (Table 3) indicate the most important variation in the ecological structure of the communities is from those in which small herbivorous species predominate to those in which large herbivores and carnivores predominate. On the basis of information referenced in the introduction, it is reasonable to expect that in lakes where small herbivores predominate smaller particles, particularly bacteria, will be grazed with attendent reduction in efficiency of utilization of primary production and planktivorous fish growth efficiency will be lower, in comparison with lakes dominated by large herbivores and carnivores which tend respectively to graze phytoplankton directly and prey on small zooplankton and are more efficiently utilized by lake planktivores. Similarly, the variation in the abundance of small carnivores summarized by the second ecological component can be expected to reflect a gradient in the intensity of invertebrate predation on small zooplankters (rotifers, copepodites) and in the efficiency with which energy passes to planktivorous fish (Kerr 1971). The third ecological component is related primarily to the relative abundance of large herbivores and hence reflects variations in fish growth efficiency (Galbraith 1975) and utilization of phytoplankton. These relations between ecological role and

TABLE 2. Structure of taxonomic principal components for Bruce lakes.

Species	Pearson correlations with species: Principal Component 1	2	3
Bosmina longirostris	0.84[a]	−0.07	0.34[b]
Diaptomus reighardi	0.43[c]	0.64[a]	−0.42[c]
Acroperus harpae	0.20	0.22	−0.07
Ceriodaphnia lacustris	0.11	−0.28	0.33[b]
Chydorus sphaericus	0.10	−0.10	0.04
Tropocyclops prasinus mexicanus	0.06	−0.41[c]	−0.14
Cyclops vernalis	0.04	−0.57[a]	0.58[a]
Leptodora kindtii	−0.06	−0.30	0.05
Mesocyclops edax	−0.09	−0.18	−0.30
Sida crystallina	−0.13	−0.02	0.14
Chaoborus sp.	−0.13	−0.18	0.24
Daphnia galeata mendotae	−0.15	0.17	0.18
Polyphemus pediculus	−0.17	0.04	0.13
Holopedium gibberum	−0.17	0.10	0.11
Cyclops bicuspidatus thomasi	−0.42[c]	0.21	0.27
Diaptomus oregonensis	−0.46[c]	−0.61[a]	−0.49[a]
Epischura lacustris	−0.47[c]	−0.02	−0.29
Daphnia retrocurva	−0.60[a]	0.04	0.09
Diaptomus minutus	−0.72[a]	0.40[c]	0.46[c]
Diaphanosoma brachyurum	−0.76[a]	0.03	0.05
Cumulative explained variance	27.1%	42.2%	55.8%

[a]Significant at 0.1% level; [b] at 5%; [c] at 1% (two-tailed test of null hypothesis of no correlation).

zooplankton body size and feeding ecology still need further investigation and clearly the magnitude as well as the efficiency of energy flow is important in practice. We wish only to emphasize that the community characterization provided by the ecological classification reflects more closely than the taxonomic characterization zooplankton community features thought to be important in lake ecosystem dynamics (Hillbricht-Ilkowska 1977).

TABLE 3. Structure of ecological principal components for Bruce lakes.

Categories	Pearson correlations with categories: Principal component 1	2	3
Herbivore 1	0.86[a]	0.08	−0.18
Herbivore 2	0.40[b]	0.08	−0.34[c]
Carnivore 1	−0.05	0.90[a]	0.09
Carnivore 2	−0.20	0.46[b]	−0.34[c]
Carnivore 3	−0.23	0.06	0.42[b]
Herbivore 4	−0.37[c]	0.11	−0.85[a]
Carnivore 4	−0.64[a]	−0.01	−0.14
Herbivore 3	−0.89[a]	−0.14	0.0
Cumulative explained variance	30.0%	55.0%	77.4%

[a]Significant at 0.1%; [b] at 1%; [c] at 5% (two-tailed test of null hypothesis of no correlation).

Many of these same functional insights emerge from patterns based on the image analysis data, which differ from the ecological data is not explicitly including feeding ecology differences and in counting smaller particles such as detritus, large phytoplankters, rotifers, and all crustacean life stages. The first image analysis component contrasts large and small organisms (Table 4) as was the case for the first ecological component. The second image analysis component is related primarily to variation in the relative abundance of particles in the 0.16–0.26 mm range. Because these two components account for an overwhelming proportion of the variation in size structure of the communities, the third component has not been included. Again these patterns in particle size distribution are likely to relate, for example, to the nature of zooplankton grazing pressures and planktivorous fish feeding efficiency and thus provide a more functional perspective of lake ecosystem structure than would taxonomic data alone.

The cumulative percentages of variation in community structure summarized by the first three components for each of the characterizations (Tables 2–4) cannot be directly compared because of differences in the number of variables (20 for taxonomy versus eight for each of the ecological and image analyses) and in their units (relative abundance for taxonomy and

TABLE 4. Structure of image analysis principal components for Bruce lakes.

	Pearson correlations with size-classes	
	Principal component	
Size-classes (mm)	1	2
<0.16	0.95[a]	−0.31[b]
0.16–0.26	0.41[c]	0.88[a]
0.26–0.36	−0.78[a]	0.32[b]
>0.83	−0.80[a]	−0.15
0.62–0.73	−0.87[a]	−0.32[b]
0.73–0.83	−0.87[a]	−0.23
0.36–0.47	−0.95[a]	−0.18
0.47–0.62	−0.97[a]	−0.15
Cumulative explained variance	77.4%	90.9%

[a]Significant at 0.1%; [b] at 5%; [c] at 1% level (two-tailed test of null hypothesis of no correlation).

ecology versus actual abundances for image analysis). Of greater importance is the comparative extent to which the patterns derived from the different community characterizations are related to other limnological and biological properties of the lakes. In this regard the ecological and image analyses are superior to the taxonomic one. The first taxonomic component is related to lake size and calcium content (Table 5) so that lakes dominated by *B. longirostris* and *D. reighardi* tend to be large and shallow with comparatively hard-water whereas those dominated by the other species (Table 2) tend to be small and deep with comparatively soft water. In total, 62% of the variation in taxonomic community structure summarized by the first component can be accounted for in terms of these limnological variables. The secondary and tertiary patterns in taxonomic structure are also related to measures of lake size which accounts for 15 and 13% of community variation, respectively.

TABLE 5. Regression of taxonomic principal components on the limnological variables.

Dependent variable	Independent variable	Regression coefficient	Coefficient of multiple determination
First principal component	Mean depth (m)	−2.35[a]	0.46
	Volume (m^3)	0.71	0.57
	Calcium ($mg \cdot L^{-1}$)	1.30	0.62
Second principal component	Lake perimeter (km)	−0.53	0.15
Third principal component	Mean breadth (km)	1.57	0.13

[a]All significance at least at 5% level.

TABLE 6. Regression of ecological principal components on the limnological variables.

Dependent variable	Independent variable	Regression coefficient	Coefficient of multiple determination
First principal component	Mean depth (m)	−8.68[a]	0.43
	Volume (m^3)	3.86	0.63
	Color	1.53	0.68
Second principal component	Volume (m^3)	6.13	0.15
	Flushing time (yr)	−7.74	0.23
	Mean breadth (km)	−10.00	0.33
Third principal component	Turbidity	1.41	0.11

[a]All significant at least at 5% level.

The first ecological component has similar relations to lake characteristics (Table 6). Lakes with predominately small zooplankters tend to be large and shallow with relatively colored water whereas those with larger species tend to be small and deep with clearer water, a pattern which supports many of the data presented by Hillbricht-Ilkowska (1977). In this case slightly more (68%) of the variation in ecological community structure summarized by the first component can be accounted for in terms of lake size, depth, and water color. As was the case for the second taxonomic component, the second ecological component is related to measures of lake size but in contrast is also related to flushing time, an important component in some production models (Dillon and Rigler 1974). Furthermore a greater percentage (33 versus 15) of the variation in community structure summarized by the second component can be assigned to the limnological variables. A small fraction of variation in the third ecological component can be assigned to changes in turbidity.

Variations in zooplankton community structure as summarized by the image analysis principal components also have strong relations to lake size and to some correlates of lake production (Table 7). The gradient along the first component from communities dominated by small to those dominated by large particles corresponds to a variation from shallow, productive (taking the inverse of mean depth times flushing rate as a correlate of production, Dillon and Rigler 1974) lakes with relatively high pH and calcium to deep unproductive and more acid lakes with lower calcium concentration. This relation to lake size and flushing rate characterizes the second image analysis component as well. With 56 and 30%, respectively, of the variation in community structure summarized by these two components attributable to measured limnological variables, the strength of these relations for the image analysis is somewhat greater than that for the taxonomic analysis.

A number of important points emerge when the re-

TABLE 7. Regression of image analysis components on the limnological variables.

Dependent variable	Independent variable	Regression coefficient	Coefficient of multiple determination
First principal component	Mean depth (m)	−0.616[a]	0.35
	(mean depth × flushing rate)$^{-1}$	1.022	0.43
	pH	0.216	0.50
	Calcium (mg·L^{-1})	0.548	0.56
Second principal component	Lake perimeter (km)	0.0352	0.08
	(flushing rate)$^{-1}$	0.0470	0.19
	shoreline dev.	0.43	0.30

[a]All significant at least at 5% level.

sults of these three different community characterizations are compared. First, eliminating taxonomic information from the characterization of zooplankton community structure has not weakened but in fact has strengthened the relations between lake type and zooplankton characteristics. The ecological and image analysis components contain all of the information about lake type that the taxonomic components contain as well as additional information not revealed by the taxonomic data (i.e. turbidity and pH for the ecological and image analysis data, respectively, and measures of lake turnover time for both). Combining the first and second components, the variation in community structure attributable to limnological variables is greatest for the ecological characterization, next for the image analysis one, and least for the taxonomic one (Tables 5–7). This result, along with earlier remarks about the greater ecological significance of particle size is the crux of our argument which can be summarized as follows. Freshwater ecologists are interested in developing predictive models about lake function, particularly with respect to energy flow. We have shown that it is possible to go from relatively easily measured hydrological and morphometric lake properties to particle size and feeding ecology characteristics of limnetic plankton communities with considerable predictive power. Insofar as these plankton characteristics are of ecological significance, it is possible to make predictions about functional aspects of lakes from simple limnological variables with some confidence. Predictions of taxonomic characteristics of plankton from these easily measured lake properties are weaker, at least partly because less easily determined parameters such as geologic history, physiological tolerances and complex competitive interactions determine taxonomic composition of plankton. At any rate, as discussed above, the aspects of taxonomy thought to be directly related to lake function are body size and feeding ecology so it only seems reasonable to emphasize these in community characterizations.

Second, it should be emphasized that although they are derived from the same data base, the ecological patterns in community structure are not mere reflections of the taxonomic patterns but actually reveal a different perspective. That is, the groups of species making up the ecological categories do not simply behave as a block in the taxonomic analysis. For instance the first taxonomic component splits *D. reighardi* from three other species (*D. oregonensis*, *D. minutus*, *Daphnia retrocurva*) which are considered as ecological equivalents and hence are combined with *D. reighardi* into the herbivore 3 category of the ecological analysis. The second ecological component is not related to the herbivorous species which correlate with the second taxonomic component. Third, despite the difference in particles counted by the ecological and image analyses (particularly the exclusion of the smallest particles from the former), the appearance of pH on the first image analysis component is the only substantive difference in limnological correlates. The ecological components have a slightly stronger relation to lake type which may indicate the value in retaining explicit recognition of feeding types as well as body size in zooplankton data.

To illustrate an additional advantage of the ecological analysis (and by implication the image analysis) over the taxonomic one, zooplankton community structure in the Bruce lakes is compared with that for lakes from Sri Lanka and the adjacent Asian mainland. It should be noted that the occurrence of littoral zooplankton species in the Asian lakes does not invalidate this comparison for littoral species are also common in the many shallow lakes with large littoral zones which are included in the Bruce survey (Table 1). Temperate and tropical regions differ considerably in their zooplankton faunas (Table 1) so that comparisons of communities on a taxonomic basis are bound to show differences. Such an analysis, however, would not indicate whether lakes in these areas differ in more functional aspects of their zooplankton communities. Analysis of patterns in size-feeding ecology community structure (Fig. 1) clearly indicates that the Asian lakes are quite distinctive from those of the Bruce Peninsula by having carnivorous species and larger species of relatively less importance. Because we hypothesize that size structure is related to energy flow, this result suggests a major functional difference of lake ecosystems in the two areas. Attempts to determine the basis for these differences in community structure have lead to hypotheses such as the greater relative importance of planktivorous fish in tropical communities (T. M. Zaret, University of Washington, Pers comm) or smaller body size of zooplankton resulting from direct physiological effects of high mean annual water temperature or indirect effects of temperature on feeding efficiency (Fernando 1979). These perceptions would have been less likely to emerge from a taxonomic comparison of the regions.

Summary and Conclusions

We wish to emphasize that, while patterns in the

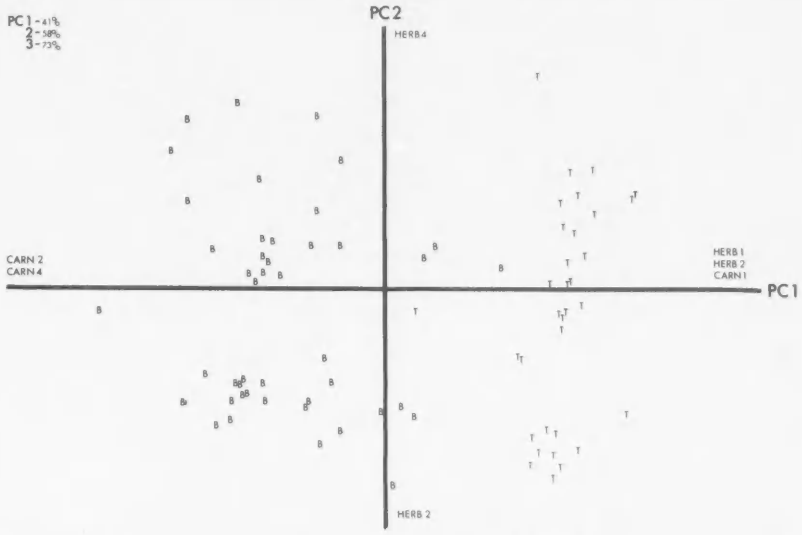

FIG. 1. Principal components ordination of the Bruce Peninsula (B's) and southeast Asian lakes (T's). Axes labels are ecological categories having statistically significant correlations with the components. The cumulative percentages of explained variance are shown for the first three components.

taxonomic structure of communities will always be of interest to the zooplankton ecologist, characterizations of zooplankton associations on the basis of body size alone or in combination with feeding ecology are of greater use in many instances because a) they much more closely reflect zooplankton community features which are related to the efficiency and nature of energy flow through pelagic ecosystems, b) their emphasis of these simpler, more ecologically significant size characteristics results in stronger relations to limnological characteristics of lakes, c) they provide a more informative comparison of plankton communities from disparate zoogeographic regions, and d) they require less money, expertise and time in community data acquisition. For activities such as lake ecosystem modelling and management which are based upon mechanisms of energy exchange, knowledge of zooplankton is important and the ecological or image analysis characterization appears to be more suitable than a taxonomic one. Particularly to laboratories engaged in programs requiring the processing of numerous plankton samples, it should be of considerable interest that the major patterns in zooplankton community structure and their relation to lake type for the Bruce lakes can be elucidated without resorting to detailed taxonomic analyses. Very roughly, individual sample processing time for the image analysis is one quarter that for the ecological analysis which is one third that for the taxonomic analysis. Excepting capital costs of major equipment and presuming one has access to an image analyzer, costs for processing individual samples follow roughly these same ratios. The taxonomic approach in particular is labor intensive.

One advantage of the ecological approach over image analysis is that published taxonomic data can be reorganized for ecological pattern analysis whereas image analysis data have to be generated de novo. For the ecological analysis, however, it would obviously be preferable to measure body size directly from zooplankton samples to obviate the necessity of using estimated or published adult sizes and to incorporate sizes of juvenile stages directly. Such analyses currently underway in our laboratory are giving promising results. The direct incorporation of juvenile sizes into the ecological and image analyses gives them the advantage of reflecting the changing ecological role of animals as they mature. Such seasonal changes in community structure have not been explicitly incorporated into this study for the Bruce lakes were sampled once only during the midsummer period. However by sampling all lakes within a comparatively short period of midsummer we have attempted to minimize the effect of seasonal changes on community patterns. The complex problem of summarizing changes in abundances of many particle sizes (or organisms) over many lakes through many time periods has been addressed by Sprules (1979b) and is currently under investigation in our laboratory.

Insofar as the image and ecological analyses are partially subjective, there is room for experimentation. The detail of feeding ecology can be increased or decreased and the range and number of size categories

can be varied. On the basis of some investigation in this regard, the schemes presented here represent an optimal approach in the statistical sense of maximum explained variance. However, there are undoubtedly sound biological bases for other modifications to the approach. For instance the inclusion of an omnivore category to better represent the tendency of cyclopoid copepods to consume both plant and animal material (Kiefer and Fryer 1978) could be considered. As more information on the feeding ecology and trophic status of zooplankters emerges modifications to the ecological classification of species can easily be made. Whatever these turn out to be, the technique described here certainly deserves attention as an alternative to taxonomically based approaches for ellucidating patterns in the structure of zooplankton communities.

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Indicators of Phosphorus and Nitrogen Deficiency in Five Algae in Culture

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HEALEY, F. P., AND L. L. HENDZEL. 1979. Indicators of phosphorus and nitrogen deficiency in five algae in culture. J. Fish. Res. Board Can. 36: 1364–1369.

Five freshwater algae (*Chlamydomonas reinhardi*, *Scenedesmus quadricauda*, *Anabaena variabilis*, *Pseudoanabaena catenata*, and *Cryptomonas erosa*) were grown in batch culture without nutrient limitation and with various degrees of P and N limitation. They were analysed for several aspects of composition and some metabolic activities of potential use as indicators of nutrient deficiency to aid in establishing values indicative of the presence or absence of nutrient deficiency. Ratios of N/C, P/C, N/P, protein/C, carbohydrate/C, and protein/carbohydrate most clearly differentiated between no, P, and N deficiency. The initial saturated rate of phosphate uptake, P debt, and alkaline phosphatase activity increased distinctly in P deficiency in some of the algae but not in others. Similarly, the initial saturated rate of ammonium uptake and N debt rose in some but not all N-deficient algae. *Cryptomonas erosa* tended to form walled resting cells (cysts) during growth into P or N deficiency.

Key words: algae, nitrogen, nutrient deficiency, nutrient uptake, phosphatase, phosphorus

HEALEY, F. P., AND L. L. HENDZEL. 1979. Indicators of phosphorus and nitrogen deficiency in five algae in culture. J. Fish. Res. Board Can. 36: 1364–1369.

Des cultures en grande quantité de cinq algues d'eau douce (*Chlamydomonas reinhardi*, *Scenedesmus quadricauda*, *Anabaena variabilis*, *Pseudoanabaena catenata* et *Cryptomonas erosa*) ont été maintenues sans limitation d'éléments nutritifs et divers degrés de limitation de P et de N. Plusieurs aspects de leur composition ont été analysés, de même que certaines activités métaboliques susceptibles de servir d'indicateurs de déficience en éléments nutritifs. Les rapports N/C, P/C, N/P, protéines/C, hydrocarbures/C et protéines/hydrocarbures sont les indicateurs les plus précis d'une absence de déficience ou d'une déficience en P ou en N. Chez certaines algues mais non chez d'autres, le taux initial d'absorption de phosphate jusqu'à saturation, la dette de P et l'activité de l'alcaline phosphatase augmentent nettement dans le cas d'une déficience en P. De même, le taux initial d'absorption d'ammonium jusqu'à saturation et la dette de N augmentent chez certaines mais non chez toutes les algues déficientes en N. *Cryptomonas erosa* tend à former des cellules au repos enkystées en milieu déficient en P ou en N.

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THE chemical composition and some metabolic activities of algae change with nutrient deficiency in ways which are qualitatively and semiquantitatively similar among algae belonging to several classes (Healey 1973b, 1975). This has prompted their use as indicators of nutrient deficiency in natural populations of algae (Haug et al. 1973; Healey and Hendzel 1976, 1979; Hitchcock 1978). To improve the application of these indicators to natural populations, values associated with the presence of various degrees of deficiency must be established using algal cultures. These studies should also show how variation among different algae under the same degree of deficiency compares with variation between degrees of deficiency. That is, are the responses of individual algal species similar enough that specific numbers can be used as an indication of the presence and absence of nutrient deficiencies in algae in general? To help in evaluating this possibility, a survey of five freshwater algae was undertaken to establish the quantitative variation in several parameters of interest at several stages of P and N deficiency.

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Materials and Methods

CULTURES AND GROWTH CONDITIONS

Pure cultures of the following five algae were used: *Chlamydomonas reinhardi* Dandeard (3, UTEX 89), *Scenedesmus quadricauda* (Turp.) de Breb. (11), *Anabaena variabilis* Kuetz (21, UTEX 1444), *Pseudoanabaena catenata* Lauterb. (57), and *Cryptomonas erosa* Ehrenbert. (40). Numbers in parentheses refer to numbers in the culture collection at the Freshwater Institute, Winnipeg, while UTEX numbers refer to the culture collection of

algae at the University of Texas. All cultures were grown in modifications of WC medium (Guillard and Lorenzen 1972). Tris buffer was replaced with 4 mM bicine to avoid interference of Tris with the determination of ammonium. Sodium nitrate was replaced with 1000 or 2000 μM NH_4Cl in complete and low P media and with 100 or 200 μM NH_4Cl in low N medium. Potassium phosphate was replaced by 50 or 100 μM KH_2PO_4 in complete and low N media and by 10 μM KH_2PO_4 and 100 μM KCl in the low P medium. Cultures were grown in 1 L of medium in large (6 × 50 cm) test tubes, bubbled with air, in a 20°C constant temperature room. The tubes were lighted from one side by Vita-Lite fluorescent tubes delivering 70–90 μEinstein $\cdot m^{-2} \cdot s^{-1}$ at the surface. Growth was measured by optical density at 750 nm.

Samples were removed for analysis during exponential growth on complete medium and at intervals following depletion of soluble reactive P (Murphy and Riley 1962) from low P medium or ammonium (Solorzano 1969) from low N medium. Generally samples were centrifuged and washed once and then resuspended in WC medium with bicine buffer but lacking N or P sources. Because *Cryptomonas erosa* was harmed by centrifuging and resuspension, metabolic measurements were made on the suspension as removed from the culture. Because this suspension was difficult to filter, a concentrated suspension for composition samples was prepared by gently centrifuging and resuspending in modified WC medium lacking N and P. Cell suspensions were used at 10–100 mg dry weight/L.

Analytical Methods

Samples for chemical composition of the algal cultures were collected by filtration on preignited (500°C) Whatman GFC filters. Cellular C, N, and P samples were washed with 10 mL modified WC medium lacking N and P and analyzed as for suspended C, N, and P (Stainton et al. 1974). Dry weight samples were dried to constant weight at 60–80°C. Protein and carbohydrate samples were taken combined. The filters containing these samples were ground in a Thomas homogenizer to prepare 5-mL suspensions. One-millilitre subsamples of these were analyzed for protein by the Lowry procedure and for carbohydrate by the anthrone procedure (Herbert et al. 1971). Lipid samples were extracted with methanol–chloroform and the extracts analyzed for C as described in Healey and Hendzel (1975). Following preextraction with 0.25 N perchloric acid at 0°C, RNA was extracted with 0.5 N perchloric acid at 70°C and measured by the phloroglucinal procedure (Herbert et al. 1971). Chlorophylls were extracted into methanol with gentle grinding, and measured spectrophotometrically using formulae for chlorophyll *a* alone or in the presence of chlorophyll *b* based on the extinction coefficients given by MacKinney (1941). Results for *C. erosa* were calculated using a formula for chlorophyll *a* in the presence of chlorophyll *c* (Jeffrey and and Haxo 1968).

Metabolic Measurements

All measurements except those of *C. erosa* were made in modified WC medium lacking N and P at pH 7.5. For *C. erosa*, the growth medium containing residual N + P was used at pH 7.5.

Alkaline phosphatase activity was measured as the rate of hydrolysis of *p*-nitrophenylphosphate (*p*NPP) to the coloured *p*-nitrophenol at 35°C (Healey 1973a). Following the addition of 2 mM final concentration *p*NPP to 20 mL of cell suspension, membrane (Sartorius cellulose nitrate 0.45 μm pore size) filtrate of culture medium, and autoclaved modified WC medium lacking N and P, 3.0-mL samples were removed at 5-min intervals over 30 min, filtered if necessary, and the optical density read at 410 nm after the addition of 3 drops of 0.1 N NaOH to ensure a pH over 8.3. Experimental rates were corrected for spontaneous hydrolysis of the substrate by subtracting the rate observed in autoclaved medium. Where activity was measured in the growth medium, the cellular activity was estimated as total minus soluble activity.

The initial rate of phosphate uptake was measured by adding a final concentration of 10 μM KH_2PO_4 to 50 mL of cell suspension. Ten-millilitre samples were removed at 5–10 min intervals and passed through Whatman GFC filters before analysis of the filtrate for soluble reactive P. Algal suspensions were lighted at 90 μEinstein $\cdot m^{-2} \cdot s^{-1}$ by fluorescent tubes at 25°C. The initial rate of ammonium uptake was measured in an analogous manner after adding a final concentration of 20 μM NH_4Cl to 50 mL of suspension in darkness at 25°C. At 5–10 min intervals over 30–60 min, 10-mL samples were filtered and analyzed for ammonium (Healey 1977).

P- and N-debts were measured by adding 10 μM KH_2PO_4 or 20 μM NH_4Cl to 100-mL algal suspensions, mixing, and removing duplicate 10-mL samples. After 24 h incubation in darkness, further duplicate samples were removed. Initial and final samples were filtered and analyzed for soluble reactive P or ammonium, the difference being interpreted as P or N debt (Healey 1973a, 1977).

Results

The results are summarized in Fig. 1–7, which compare several aspects of the composition and metabolism of the five algae studied as a function of growth rate limited to known extents by N or P deficiency. The figures also compare the present results with the results of a literature survey of the effects of nutrient deficiency on algae (Healey 1975). This survey showed a large degree of similarity, both qualitative and quantitative, between a wide variety of algae in their responses to nutrient deficiency, primarily N and P deficiency. On the basis of this survey, division points between the presence of no deficiency (exponentially-growing algae,) moderate deficiency (a growth rate of about half the maximum), and extreme deficiency (a growth rate of less than one-fifth the maximum) were suggested. These were converted from a dry weight basis to an organic C basis assuming C to be 50% of dry weight, and are indicated on the figures by horizontal broken lines. The symbols in the right-hand column indicate ranges which this literature survey associated with no (N), moderate (M), and extreme (E) N or P deficiency or the presence or absence of N or P deficiency.

Composition

The ratio of dry weight/C was not affected by N or P deficiency and averaged about 2000 μg/mg C in all the algae studied (Fig. 1). The range of values in Fig. 1

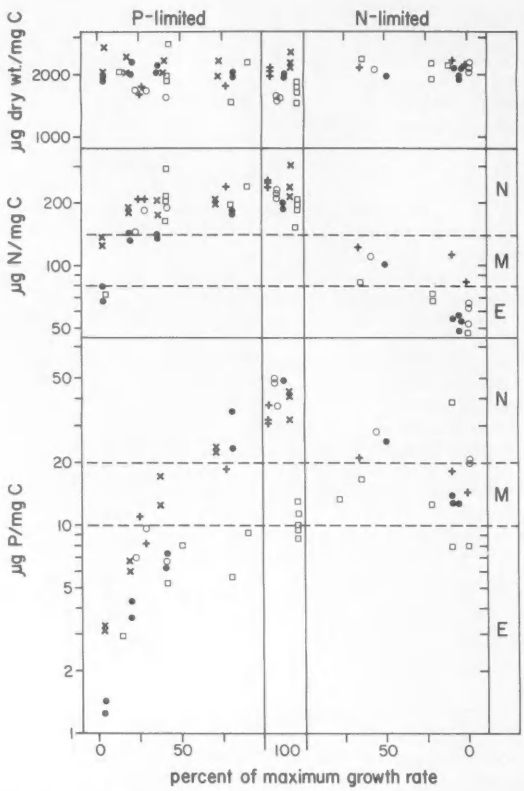

FIG. 1. Particulate dry weight, N, and P per unit particulate C as a function of P- and N-limited growth rates. (*Scenedesmus quadricauda* (●), *Chlamydomonas reinhardi* (○), *Anabaena variabilis* (×), *Pseudoanabaena catenata* (+), *Cryptomonas erosa* (□). Horizontal broken lines indicate division points between no (N), moderate (M), and extreme (E) N or P deficiency based on a literature survey (Healey 1975).

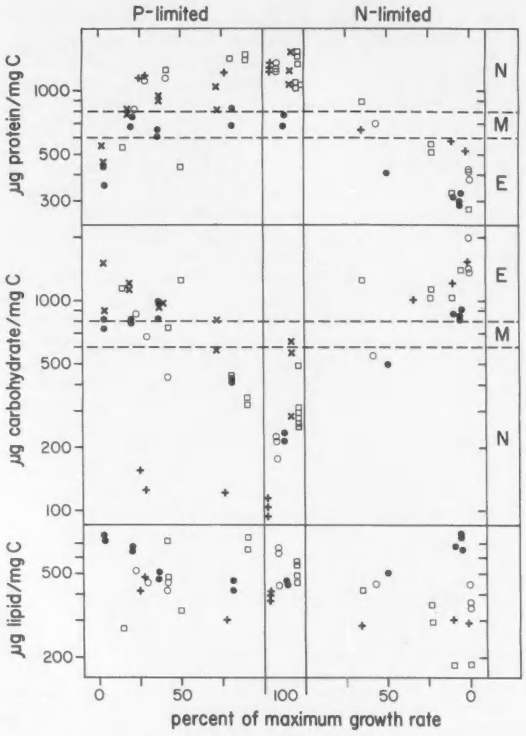

FIG. 2. Particulate protein, carbohydrate, and lipid per unit particulate C as a function of P- and N-limited growth rates. Symbols as in Fig. 1.

is the same as that reported in the literature for a variety of algae (Healey 1975). The N/C ratio was quite similar in all five species across the range of conditions studied (Fig. 1). While this ratio fell progressively as N deficiency increased, it was much less affected by P deficiency, falling only in severe P deficiency to values found under N deficiency. The P/C ratio showed greater spread, primarily because of the low values found in *C. erosa* (Fig. 1). The P/C ratio of exponential cells of this alga was low enough to fall in the range associated with moderate P deficiency in other algae. In all five species, this ratio fell progressively and steeply with P deficiency and to a much lesser degree with N deficiency. Combining these components, the N/P ratio (Fig. 4) showed little change between N-limited and exponential cells, as seen elsewhere (Healey 1975), but rose sharply into P-limitation in four of the species. *Cryptomonas erosa*, on the other hand, showed a sharp rise in N/P ratio between N- and no limitation with little further increase into P-limitation.

The protein/C ratios of four of the species were for the most part quite similar to one another (Fig. 2). The values for *S. quadricauda* were noticeably lower, especially in exponential cells. The pattern with deficiency was similar to that of the N/C ratio, falling progressively with N deficiency and with P deficiency only at the lower growth rates. Carbohydrate/C showed approximately the inverse pattern (Fig. 2), rising in a similar manner in both N and P deficiency. An exception was *P. catenata* which showed marked accumulation of carbohydrate in N deficiency but very little in P deficiency. The large variation in carbohydrate/C among exponentially-growing algae covers the range reported elsewhere (Healey 1975). Since these two parameters varied in opposite directions, combining them in the protein/carbohydrate ratio could be expected to show even greater changes with both N and P deficiency. Figure 4 shows this ratio dropping somewhat more sharply in N than in P deficiency. While there was a wide range of values between the various species, the trends were similar and there was great enough

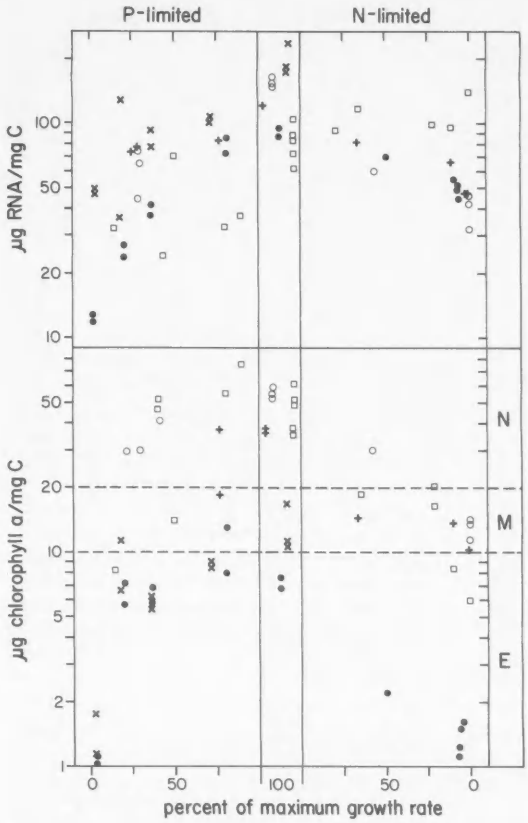

FIG. 3. Particulate RNA and chlorophyll *a* per unit particulate C as a function of P- and N-limited growth rates. Symbols as in Fig. 1.

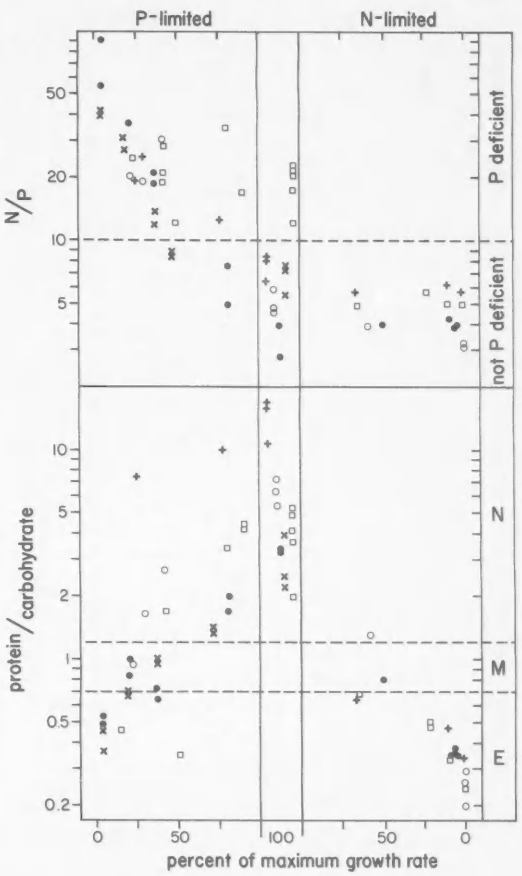

FIG. 4. Weight ratios of N/P and protein/carbohydrate as a function of P- and N-limited growth rates. Symbols as in Fig. 1.

variation for cells of various degrees of deficiency to be differentiated. *Pseudoanabaena catenata* was again an exception, showing little decline in this ratio in P deficiency because it did not accumulate carbohydrate. Viewed overall, lipid/C (Fig. 2) showed no variation with N or P deficiency but patterns were evident, varying from one algae to another. *Scenedesmus quadricauda* showed lipid accumulation in both severe N and severe P deficiency. *Cryptomonas erosa* showed the opposite pattern, having a lowered lipid content in both P and N deficiency. *Chlamydomonas reinhardi* and *P. catenata* showed little variation with N or P deficiency.

Both RNA/C and chlorophyll *a*/C ratios showed greater variation from species to species than the preceding parameters (Fig. 3). Thus, while RNA/C fell with P and N deficiency in all situations tested (except *C. erosa* which showed no drop in N deficiency), the range of ratios of exponential cells overlapped that of moderately and even severely limited cells. In the published literature, the overlap between deficient and nondeficient algae was too great to suggest division points between the presence and absence of deficiency (Healey 1975). The chlorophyll *a*/C ratio was similar to N/C and protein/C, declining progressively into N deficiency and less so into P deficiency, to fall sharply only in severe P deficiency. The chlorophyll *a*/C ratio of *S. quadricauda* and *A. variabilis*, like that of some other algae (Healey 1975), was low enough in exponential cells to be in the range expected for P- or N-deficient algae.

METABOLISM

The three metabolic parameters responding to P deficiency, the initial saturated rate of phosphate uptake, the P debt, and cell surface alkaline phosphatase activity, all showed similar patterns (Fig. 5). Activities were

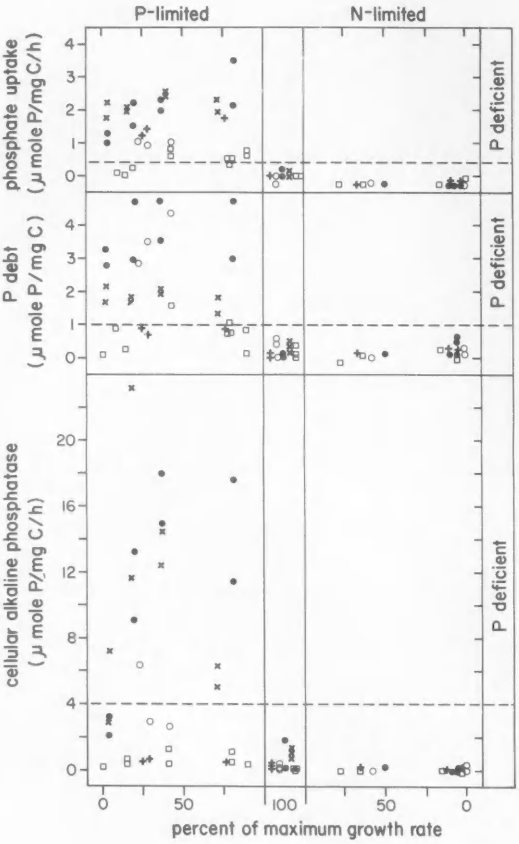

FIG. 5. Initial saturated rate of phosphate uptake, P debt, and alkaline phosphatase activity as a function of P- and N-limited growth rates. Symbols as in Fig. 1. Values above the horizontal broken lines associated with the presence of P deficiency and those below with the absence of P deficiency on the basis of published literature (Healey 1975).

low and similar in exponential and N-limited cells and tended to rise in P deficiency. However, the extent of this rise was by no means uniform among the species. Some showed a sharp rise so the presence of P-limitation was clearly distinct from its absence (*S. quadricauda*, *C. reinhardi*, *A. variabilis*). *Pseudoanabaena catenata* showed an increase in the rate of phosphate uptake and the size of the P debt, but the latter was not clearly separated from the values of other algae when P-sufficient. This alga showed no rise in alkaline phosphatase activity. *Cryptomonas erosa* showed small rises in all three reactions at P-limited growth rates over 50% of the maximum but these were not clearly separated from values of other algae when not P-limited and declined as P deficiency increased further.

Two metabolic indicators of N deficiency showed the

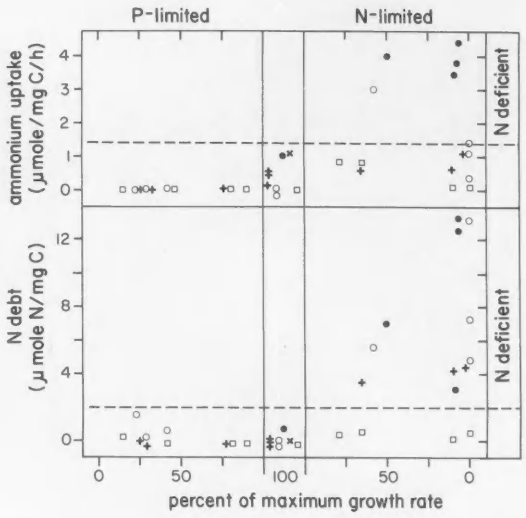

FIG. 6. Initial saturated rate of ammonium uptake and N debt as a function of P- and N-limited growth rates. Symbols as in Fig. 1. Values above the horizontal broken lines associated with the presence of N deficiency and those below with the absence of N deficiency on the basis of published literature (Healey 1975).

opposite pattern tending to rise more or less in N deficiency from the low values found in P-limited and exponential cells (Fig. 6). Both the initial saturated rate of ammonium uptake in darkness and the N debt rose clearly in *S. quadricauda* and *C. reinhardi*. *Pseudoanabaena catenata* showed a lesser increase especially in the rate of ammonium uptake, while *C. erosa* showed little change.

Cryptomonas erosa showed a marked morphological change during growth into nutrient deficiency. Formation of cysts (round, nonmotile, apparently thick-walled cells) increased with both N and P deficiencies (Fig. 7). If these cysts were low in metabolic activity, their formation may account for the failure of metabolic indicators to increase in nutrient deficiency in this alga.

Discussion

The present results show clearly that some parameters are better indicators of N or P deficiency than are others, showing either greater differences between various stages of deficiency and/or less scatter for a given degree of deficiency. In this sense, the carbohydrate/C and protein/carbohydrate ratios are good indicators of both P and N deficiency, the P/C and N/P ratios of P deficiency, and the N/C and protein/C ratios of N deficiency. While the chlorophyll *a*/C ratio showed a large decline into N deficiency and a lesser decline into P deficiency, the scatter from one alga to another found

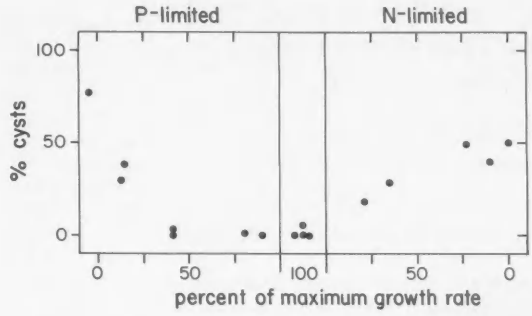

FIG. 7. Percentage of *Cryptomonas erosa* cells which are cysts as a function of P- and N-limited growth rate.

here makes it a less useful indicator. The RNA/C and lipid/C ratios offer little promise of distinguishing P and N deficiencies. The metabolic indicators (uptake rates, nutrient debts, and alkaline phosphatase activities) were all similar in being sensitive indicators of N or P deficiency in some algae but very insensitive in others. This variation in usefulness among the several potential indicators emphasizes the need for more than one indicator to be measured when the nutrient status of a natural population is evaluated. The results also show that while the presence of nutrient deficiency symptoms are clear evidence of the presence of that deficiency, the absence of some symptoms does not always mean the absence of deficiency. The high chlorophyll *a* content of some P-deficient algae and the low phosphate uptake rates, P debts and alkaline phosphatase activities of other P-deficient algae provide examples of this.

While most of the algae examined here behaved in similar manners, there were some exceptions. *Cryptomonas erosa* differed from the other algae surveyed here and elsewhere (Healey 1975) in several ways. This is a reminder that the uniformity of responses among algae reported in the literature (Healey 1975) is based largely on studies on green and blue-green algae and diatoms. While these are the most common groups of algae in eutrophic situations, more observations on cryptomonads, chrysophytes, and dinoflagellates are needed.

There is general agreement between the present measurements and the results of a previous literature survey (Healey 1975) both in the trends and in the values found. This supports the generality of these parameters as indicators of N and P deficiency and reinforces the values established earlier as division points between the presence and absence of N and P deficiency. Thus, to determine the nutritional status of a natural population, it does not seem necessary to study the responses to nutrient deficiency of the individual major components of the population. Rather, it is sufficient to compare the properties of the natural population with values indicative of the degree of deficiency based on results from a variety of algae.

Acknowledgments

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Bacterial Kidney Disease in Feral Populations of Brook Trout (*Salvelinus fontinalis*), Brown Trout (*Salmo trutta*), and Rainbow Trout (*Salmo gairdneri*)

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MITCHUM, D. L., L. E. SHERMAN, AND G. T. BAXTER. 1979. Bacterial kidney disease in feral populations of brook trout (*Salvelinus fontinalis*), brown trout (*Salmo trutta*), and rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 36: 1370–1376.

Incidence and effects of bacterial kidney disease (BKD) were determined in wild, naturally reproducing populations of brook trout (*Salvelinus fontinalis*), brown trout (*Salmo trutta*), and rainbow trout (*Salmo gairdneri*) in a small lake and stream system in southeastern Wyoming, USA where BKD epizootics have been observed since 1972. During 1976, dead fish were collected at three upstream stations, and 60 live fish were collected from each of 11 stations. All fish were necropsied, and virological, bacteriological, and parasitological examinations were conducted by standard methods. An indirect fluorescent antibody technique was used to detect the BKD organism in cultures and kidney tissue smears. Bacterial kidney disease was diagnosed in 100% of the dead brook trout collected. Incidence among live fish ranged from 83% at an upstream station to only 3% at the most downstream location, and was highest in brook trout and lowest in rainbow trout. Two longnose suckers (*Catostomus catostomus*), the only non-salmonids collected, were found negative for BKD. Clinical signs of infection and the most severe infections were found only in brook trout. Five age-classes of feral brook trout were involved in the epizootics. Since other known pathogens were essentially absent, it is believed that all deaths were due to BKD. Relationships between species susceptibility to BKD, age-classes, water chemistry and water temperatures, and certain ecological conditions are discussed.

Key words: bacterial kidney disease, feral trout, epizootics, brook trout, brown trout, rainbow trout

MITCHUM, D. L., L. E. SHERMAN, AND G. T. BAXTER. 1979. Bacterial kidney disease in feral populations of brook trout (*Salvelinus fontinalis*), brown trout (*Salmo trutta*), and rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 36: 1370–1376.

L'occurrence et les effets de la maladie bactérienne du rein (BKD) ont été déterminés chez des populations sauvages, se reproduisant naturellement, d'ombles de fontaine (*Salvelinus fontinalis*), de truites brunes (*Salmo trutta*) et de truites arc-en-ciel (*Salmo gairdneri*) dans un réseau comprenant un petit lac et un cours d'eau dans le sud-est du Wyoming, E.-U., où l'on observe des épizooties de BKD depuis 1972. En 1976, on a recueilli des poissons morts à trois stations du haut du cours d'eau et capturé 60 poissons vivants à chacune de 11 stations. On a fait l'autopsie de tous les poissons et effectué, selon les techniques ordinaires, des examens virologiques, bactériologiques et parasitologiques. Une technique avec anticorps à fluorescence indirecte a été utilisée pour détecter l'organisme de la BKD dans des cultures et sur des frottis de tissu rénal. On a diagnostiqué la maladie bactérienne du rein chez 100% des ombles de fontaine recueillis morts. Chez les poissons capturés vivants, l'occurrence varie de 83% à une station d'amont à seulement 3% à la station la plus en aval; elle est maximale chez les ombles de fontaine et minimale chez les truites arc-en-ciel. Deux meuniers rouges (*Catostomus catostomus*), les seuls non-salmonidés capturés, ne sont pas affectés par la BKD. On observe chez les ombles de fontaine seulement des signes cliniques d'infection, de même que les plus sérieuses infections. Les épizooties affectent cinq classes d'âge d'ombles de fontaine sauvages. En l'absence presque complète d'autres agents pathogènes connus, on croit que toutes les mortalités sont dues à la BKD. On examine les relations entre la susceptibilité spécifique à la BKD, les classes d'âge, la chimie et la température de l'eau, d'une part, et certaines conditions écologiques, d'autre part.

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BACTERIAL kidney disease (BKD) is a systemic infection of salmonid fish caused by an organism with the proposed name *Corynebacterium salmoninus* (Sanders and Fryer 1978). The disease cannot be cured by chemotherapy (Snieszko and Griffin 1955; Wolf and Dunbar 1959a; Warren 1963a). The course of the disease is usually chronic, but acute outbreaks do occur (Bullock et al. 1975).

Most records or studies on the occurrence of BKD have been made in hatcheries or laboratories. The disease has also been reported in wild or apparently wild Atlantic salmon (*Salmo salar*) in Scotland (work cited by Smith 1964) and in Canada (Pippy 1969); in chinook salmon (*Oncorhynchus tshawytscha*) in the United States (Wood and Wallis 1955; Ellis et al. 1978); in coho salmon (*O. kisutch*) in the United States (MacLean and Yoder 1970); and in rainbow trout (*Salmo gairdneri*) in Canada (Evelyn et al. 1973). Except for apparent regular outbreaks of BKD in Pacific and Atlantic salmon, reports of regularly occurring BKD epizootics in wild populations were not found. Also, we are not aware of any extended studies on BKD in feral populations of fish.

During August 1972 BKD was diagnosed in a wild, naturally reproducing brook trout (*Salvelinus fontinalis*) population in North French Creek, Carbon County, Wyoming (Mitchum 1972). This stream and associated tributaries are located in southeastern Wyoming at elevations ranging from about 3170 m (headwaters) to 2470 m. The source of the disease is thought to have been infected hatchery trout stocked into the stream from a station with a history of BKD and the only hatchery ever to have provided fish for the creek. No stocking has occurred since 1963. Epizootics have been observed each year since 1972. This situation provided an excellent opportunity to investigate BKD under natural conditions. The study was initiated in 1976 to determine the incidence and effects of the disease in the feral trout populations in the French Creek–Brush Creek drainage.

Materials and Methods

Eleven sampling stations were established on the drainage system to collect fish for examination (Fig. 1). This study area included one 4.8-ha lake (Station 1) and about 88 km of various creek tributaries. Dead fish were collected at the three upstream Stations 1, 2, and 3 (area of known epizootics) twice a week during July through mid-October 1976. Also, a sample of 60 live fish was collected with gill nets from Station 1, and with electrofishing gear from each of the other 10 stations (Ossiander and Wedemeyer 1973). There were actually two collection sites (A and B) for Stations 6, 10, and 11. At these stations approximately one-half of the live fish sample was collected at Site A and one-half at Site B, giving a total of 60 fish. This was done to expand the actual areas surveyed in these streams.

All collected fish were necropsied, and virological, bacteriological, and parasitological examinations were conducted by standard methods (AFS/FHS 1974). Kidney and occasionally liver and spleen were cultured on KDM-2

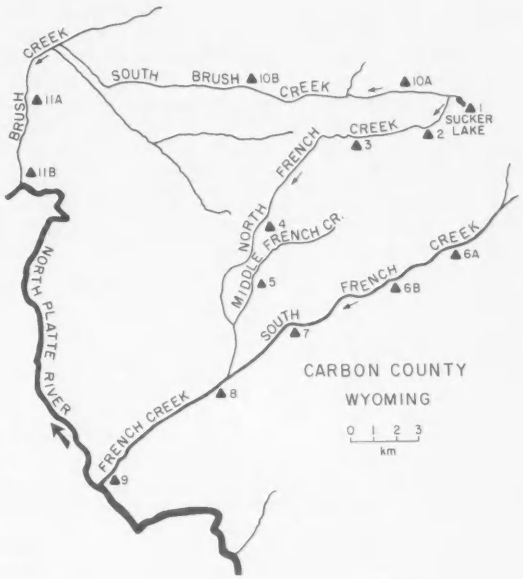

FIG. 1. French Creek–Brush Creek drainage system showing locations of sampling stations.

(Evelyn 1977). An indirect fluorescent antibody technique (FAT) was used to detect *C. salmoninus* in cultures and in kidney tissue smears (Bullock and Stuckey 1975). Smears were examined at 1500× on an American Optical Series 20 microscope using incident-light from an Osram HBO 50-W lamp with a BG12 + KV418 exciter filter and 500-nm dichroic beamsplitter element in combination with an OG515 barrier filter.

The severity of BKD infection in the dead and live trout collected from the 11 sampling stations was determined. Criteria for assigning numerical indices of severity using the indirect FAT were modified after Lewis and Allison 1971.

Major ion concentrations, total dissolved solids, and hardness were determined by standard methods (APHA et al. 1971) for water samples collected at each of the 11 stations. Maximum–minimum water temperatures were also recorded twice a week during July through mid-October at Stations 1, 2, and 3.

The age-classes of the 1976 fish samples collected from Stations 2 and 3 were determined by matching the sizes of the 1976 fish with those of known age from 1973 samples. The latter were aged by the scale method. Spot checks of scales from representative 1976 fish were made to confirm age-class estimates. Because of continued similar environmental conditions, it is believed that the rate of growth did not change significantly between 1973 and 1976.

Results and Discussion

Bacterial kidney disease was diagnosed in all dead feral brook trout collected from the three upstream Stations 1, 2, and 3 (Table 1). Of the 60 live fish collected

TABLE 1. Incidence of bacterial kidney disease in feral trout collected from 11 stations on the French Creek–Brush Creek drainage system.

Stn. No.	BKD positive/sample size (% positive)[a]				
		Live fish[b]			
	Mortalities (all brook trout)	Brook trout[c]	Brown trout	Rainbow trout	All Species
1	45/45	36/60	—	—	(60%)
2	20/20	13/45	—	—	(29%)
3	143/143	46/58	—	—	(79%)
4	—	50/60	—	—	(83%)
5	—	15/60	—	—	(25%)
6	—	5/30	8/30	—	(22%)
7	—	5/24	15/36	—	(33%)
8	—	1/5	17/52	1/3	(32%)
9	—	0/2	5/29	4/29	(15%)
10	—	10/60	—	—	(17%)
11	—	1/26	1/27	0/7	(3%)

[a]Incidence of the BKD organism was determined in kidney tissue by the indirect fluorescent antibody technique of Bullock and Stuckey (1975).

[b]Live trout from Station No. 1 (a lake) were collected with gill nets; live trout from the other stations (all streams) were collected with electrofishing gear.

[c]A total of only 45 and 58 live brook trout was collected from Stations 2 and 3, respectively. Sixty live trout were collected from each of the other stations.

from each of the 11 stations, incidence of BKD ranged from 83% at an upstream station to only 3% at the most downstream location. Only brook trout were obtained at upstream Stations 1–5 and 10, but brown trout (*Salmo trutta*) and rainbow trout were most commonly collected at the downstream stations (Table 1, Fig. 1). Bacterial kidney disease was found in all three trout species with the overall incidence highest in the brook trout, lower in brown trout, and lowest in rainbow trout. These data on relative susceptibility among the species agree with those of Bullock et al. (1971). However, at stations 6 and 7 where brook trout and brown trout were collected and at stations 8 and 9 where all three species were collected, incidence of BKD was lowest in brook trout. This apparent contradiction may be due to the small sample sizes. Continuing research should resolve this question of relative susceptibility of these trout species.

The most severe infections (4+ and 3+) occurred primarily in dead trout collected from the upstream locations (Table 2, Fig. 1). Clinical signs (light-colored and swollen kidneys with typical grayish-white necrotic lesions) were observed only in brook trout collected from the five most upstream locations (Stations 1–4 and 10, Fig. 1). The area encompassing Stations 1, 2, and 3 was determined to be the focus of infection because of the deaths which occurred there, and because of the high incidence and severity of BKD in the trout examined from these locations. Most of the infected live trout collected from the downstream stations had low severity (1+) infections. Trout with low severity infections (1+ and 2+) can probably be classified as BKD carriers or recently infected fish.

Two mature male longnose suckers (*Catostomus catostomus*), the only nonsalmonids collected, were gill netted from Sucker Lake (Station 1). Both specimens were found to be negative for BKD, even though the incidence and severity of BKD was high in the trout collected from Station 1 (Tables 1 and 2). This finding supports other accounts (Bullock et al. 1971; AFS/FHS 1974; Sanders and Fryer 1978) that the occurrence of BKD among nonsalmonids is unknown.

Except for an occasional secondary fungus infection (*Saprolegnia* sp.) in some trout mortalities, no parasitic or viral diseases were found, but in addition to the BKD organism, 11 other bacterial species were isolated from dead feral brook trout (Table 3). Because the BKD organism is difficult to cultivate, it was not isolated in all specimens cultured. It is most likely that *C. salmoninus* was lost because of overgrowth by some of the other listed bacteria. However, the BKD organism was found by FAT in all of the mortalities (Table 1). Since other known fish pathogens were absent or found only occasionally in BKD infected fish (Table 3), it is believed that all of the deaths were due to BKD.

The total dissolved solids and water hardness levels were low (usually less than 100 mg/L) at all sampling stations. Waters at Stations 1, 2, and 3, where annual BKD epizootics occur, were very low in dissolved solids (8.7 mg/L average) and hardness (4.7 mg/L average). Warren (1963b) found that in hatchery fish, BKD was most severe at hatcheries having water low in hardness (9.9 mg/L average) and either absent or of much less severity at hardwater stations (311.9 mg/L average).

The relationship between the softer waters present at upstream Stations 1, 2, and 3 and the higher incidence

TABLE 2. Severity of bacterial kidney disease in dead and live feral trout collected from 11 stations on the French Creek–Brush Creek drainage system.

Stn. no.	Severity of infection[a]									
	0 (negative)		1+		2+		3+		4+	
	Dead	Live	Dead	Live	Dead	Live	Dead	Live	Dead	Live
1	0/45	25/60	6/45	21/60	3/45	9/60	2/45	3/60	34/45	2/60
2	0/20	32/45	5/20	8/45	8/20	4/45	3/20	1/45	4/20	0/45
3	0/143	12/58	18/143	23/58	4/143	18/58	1/143	4/58	120/143	1/58
4	—	10/60	—	28/60	—	20/60	—	2/60	—	0/60
5	—	45/60	—	11/60	—	4/60	—	0/60	—	0/60
6	—	47/60	—	13/60	—	0/60	—	0/60	—	0/60
7	—	40/60	—	20/60	—	0/60	—	0/60	—	0/60
8	—	41/60	—	19/60	—	0/60	—	0/60	—	0/60
9	—	51/60	—	9/60	—	0/60	—	0/60	—	0/60
10	—	50/60	—	9/60	—	0/60	—	0/60	—	1/60
11	—	58/60	—	2/60	—	0/60	—	0/60	—	0/60

[a]Criteria for the assignment of numerical indices of BKD severity using the indirect fluorescent antibody technique were modified after Lewis and Allison 1971. (0 (negative) = fluorescent cells not observed in 50 fields; 1+ = average of 1 to 2 fluorescent cells per 25 fields; 2+ = average of 1 to 2 fluorescent cells per 10 fields; 3+ = average of 1 to 2 fluorescent cells per field; 4+ = 3 or more fluorescent cells per field.)

and severity of BKD found there, compared to the downstream stations, may be important. However, as Warren (1963b) stated in his hatchery fish survey, other factors in addition to, or in combination with hardness must be considered. In our study these considerations would include environmental factors (stream and lake ecology) and the relative susceptibility or resistance to BKD of the trout species present at the various stations (Table 1).

Ecological conditions are somewhat different at the various sampling stations in the study area, and they probably affect the incidence and severity of BKD. For example, downstream stations have a greater water flow than the headwater stations. Perhaps in these higher volumes and velocities of water flow, the BKD organism, shed from infected fish, becomes diluted to a point unfavorable for its transmission to new host fish. Also, as was noted earlier, brown trout and rainbow trout are more numerous than brook trout at these downstream stations, and they may have more natural resistance to BKD as stated by Bullock et al. (1971). Sucker Lake (Station 1) is drained annually during the fall to only about one-fourth of its normal volume to provide irrigation water for downstream ranches. Although no increase in mortalities was evident during this low water period, the situation probably causes some stress to the fish population. The presence and number of pools in the streams may also be an important factor to consider. The water quality is similar at Stations 1, 2, 3, and 10. These are all closely situated upstream stations (Fig. 1) in which only brook trout occur. However, the incidence and severity of BKD at Station

TABLE 3. Bacterial species isolated from feral brook trout mortalities collected from three stations on the French Creek–Brush Creek drainage system.

Bacterial species	Frequency isolated/BKD organism also cultured			
	Station No. 1 (21 cultured)	Station No. 2 (10 cultured)	Station No. 3 (76 cultured)	Total
Lactobacillus sp.	1/1	2/1	18/13	21/15
Citrobacter freundii	5	1	3/2	9/2
Arizona sp.	2/1	3/1	2	7/2
Escherichia coli	4	0	2	6
Pseudomonas fluorescens	2	1	3	6
P. alcaligenes	0	1	4	5
Aeromonas hydrophila	0	4	0	4
Acinetobacter sp.	0	1	2	3
Achromobacter sp.	0	0	2	2
Enterobacter aerogenes	0	1	0	1
Pectobacterium sp.	1	0	0	1

10 was found to be much less than at Stations 1, 2, and 3 (Tables 1 and 2). This difference could be due to different ecological conditions. Station 10 (A and B) has a greater water flow than Station 2 but less than Station 3. An important difference between Station 10 and Stations 2 and 3 may be the fact that no significant pool areas are present at Station 10 in contrast to the occurrence of many pools at Stations 2 and 3. Congregations of fish were often observed in these pool areas. Perhaps fish-to-fish transmission of BKD is facilitated by pool-type habitat. Exposure of host fish to contaminated feces may be greater in these pools as compared to the more shallow, swifter flowing waters. Presence of the BKD organism in feces collected from North French Creek trout during 1977 was demonstrated by using the FAT (unpublished data).

Mortalities were observed at Stations 1–3 as soon as the streams were accessible on July 2, 1976 through the last collection date on October 12, 1976. Mean water temperatures during this period ranged between 2.8°C in mid-October to 17°C in mid-July with light mortalities occurring during the entire period. Peak mortalities tended to follow peaks in water temperatures (Fig. 2A and C). Sanders et al. (1978) found in experimentally BKD-infected coho salmon and steelhead trout (*Salmo gairdneri*), but not in sockeye salmon (*Oncorhynchus nerka*), that higher water temperatures (17.8 and 20.5°C as opposed to 3.9 to 15°C) suppressed development of fatal disease; but fish which did develop fatal infections showed the shortest survival time at the higher temperatures. Wood (1974) indicated that BKD mortalities in Pacific salmon occur more rapidly at higher temperatures (above 11.1°C) than at lower temperatures (7.2–10°C). Bullock et al. (1971) stated that BKD epizootics can occur at temperatures of 10°C or below, but that the course of the disease may be accelerated and mortalities heavy during higher temperatures (13–18°C). However, Wolf and Dunbar (1959b) observed greater losses in experimentally BKD-infected brook trout at 7°C than at 12.5°C. Data obtained in our study support the general findings of others in that BKD causes mortalities in salmonids over a wide temperature range.

Five age-classes (0–IV) of feral brook trout were found at Stations 2 and 3, the only stations where ages of the collected fish were determined (Table 4). Age-classes II and III predominated in both live fish and mortality categories. Bacterial kidney disease was found in all age-classes including two age-class 0 specimens (Table 4). Brook trout eggs hatch during late March through May in North French Creek. The two infected age-class 0 specimens were collected during July (Station 2) and September (Station 3). Therefore, natural infections of BKD in feral brook trout may occur vertically through the egg, or the fish can become infected horizontally from other infected fish at 2–6 mo of age or less. Although acute forms of BKD occur, the disease is more typically chronic and seldom occurs in fish less than 6 mo old (AFS/FHS 1974). Wood (1974)

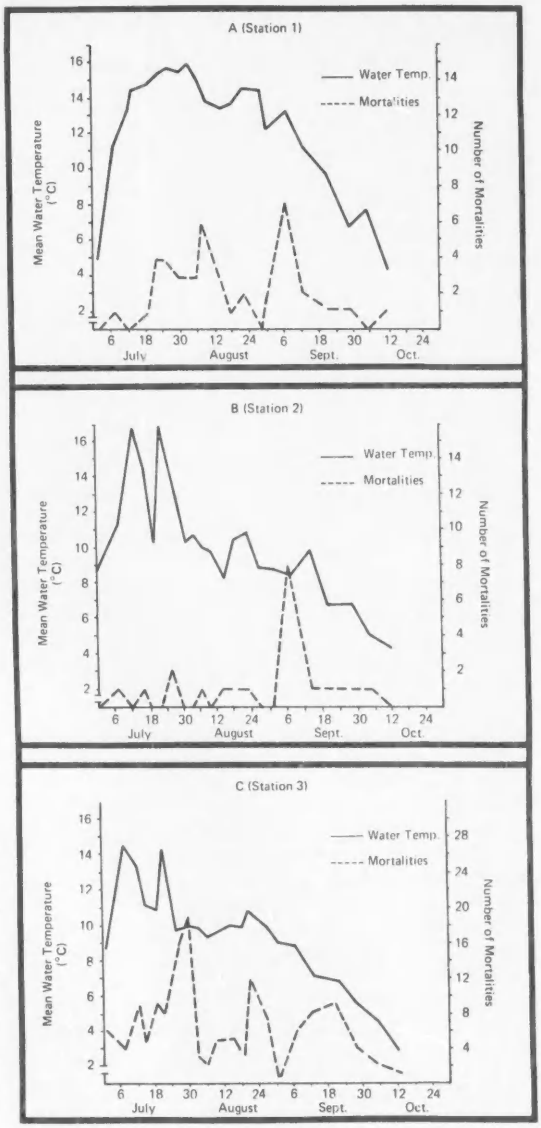

FIG. 2. Water temperatures and numbers of feral brook trout mortalities for three stations on the French Creek–Brush Creek drainage system.

indicated that, depending on water temperature and intensity of exposure, losses from BKD can occur in 1–3 mo in Pacific salmon. In experiments conducted by Wolf and Dunbar (1959b) mortalities due to BKD occurred between 48 d (about 1.6 mo) and 102 d (about 3.4 mo) in brook trout whose skin had been manually abraded and exposed to the causative organism.

No fish older than age-class IV were collected (Table

TABLE 4. Incidence of bacterial kidney disease according to age-class in feral brook trout collected from two stations on North French Creek.

Stn. no.	Age 0		Age I		Age II		Age III		Age IV	
	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.
					Mortalities					
2	0	0	4	0	7	0	8	0	1	0
3	1	0	14	0	49	0	65	0	14	0
					Live fish					
2	1	0	2	8	10	15	0	7	0	2
3	0	0	9	0	14	3	17	7	6	2

4), and there was a significant decrease in numbers of fish collected from the oldest age group (age-class IV) as compared to numbers collected from the next oldest age groups (age-classes II and III). This could indicate simply that older fish die from other causes, e.g. old age, angler harvest, or animal predation. However, it is also possible that the older fish in the populations are being depleted by BKD. The incidence of BKD is high in all age groups (Table 4), but necropsies and FAT conducted during this study show that the most severe infections occurred predominately in fish in the older age-classes.

In summation, it appears that a combination of ecological conditions is most important in influencing the distribution of BKD in the French Creek–Brush Creek drainage system. These conditions include the lower volumes and velocities of water, the occurrence of many pools, and the softer waters present at the upstream stations; and stress due to severe annual water fluctuation in Sucker Lake (Station 1). We also believe the host species present at the various stations, although important, is secondary, in explaining distribution of the disease. However, the high incidence and severity of infection in upstream brook trout is considered a definite factor effecting perpetuation of the disease in the entire drainage system.

Data obtained during our study of BKD in feral populations of trout add to the knowledge of the disease and are in general agreement with other reported observations. Studies on the BKD problem in the North French Creek–Brush Creek drainage system are continuing. These include investigations to determine if newly stocked hatchery salmonids become infected with BKD and to determine the relative susceptibility of different species to the disease. Also, an attempt is being made to control or eradicate BKD in the drainage system by reducing or eliminating the existing heavily infected upstream brook trout populations via chemical treatment. Results of these studies may aid fishery workers in devising plans to control or prevent any future BKD epizootics and to control fish diseases in general.

Acknowledgments

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Differential CO_2 and O_2 Benthic Community Metabolism in a Soft-Water Lake

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RICH, P. H. 1979. Differential CO_2 and O_2 benthic community metabolism in a soft-water lake. J. Fish. Res. Board Can. 36: 1377–1389.

CO_2 accumulation is greater than O_2 removal in the hypolimnion of Dunham Pond (z_{max} = 4.5 m) during the summer, and in the whole lake during winter stratification. During much of stratification no free oxygen and less than 1% surface light exist below 2.5 m. Despite low light and on-going accumulation of CO_2 during stratification, CO_2 uptake is found in situ in sediment surface incubations at 3.5 m. The CO_2 uptake is associated with an epipelic mat of blue-green algae apparently undergoing anaerobic photosynthesis. Thus, anaerobic photosynthesis and anaerobic respiration appear to be going on simultaneously in the hypolimnion. Respiratory quotients are conventionally thought of as a characteristic of metabolism imparted by the nature of the biochemical substrate being oxidized by an organism. Respiratory quotients of mixed communities of aerobes and anaerobes depend more upon the nature of the oxidizing agent being reduced. The degree to which a community RQ is greater than expected (about 0.85) describes the amount of metabolism for which oxygen is not (yet) the terminal electron acceptor. Excess oxidation of carbon creates ambient reducing power potentially available for bacterial (anaerobic) photosynthesis and chemolithotrophy.

Key words: lake, respiratory quotient, oxygen deficit, carbon cycle, benthic respiration, eddy diffusion, heat budget, soft-water, detritus, ecosystem energetics

RICH, P. H. 1979. Differential CO_2 and O_2 benthic community metabolism in a soft-water lake. J. Fish. Res. Board Can. 36: 1377–1389.

L'accumulation de CO_2 est plus grande que la perte d'O_2 dans l'hypolimnion de l'étang Dunham (z_{max} = 4,5 m) en été et dans tout le lac durant la période de stratification hivernale. Pendant une bonne partie de cette dernière, il n'existe pas d'oxygène libre et, au-delà de 2,5 m de profondeur, il y a moins de 1% de l'éclairement de surface. En dépit de la faible intensité lumineuse et de l'accumulation continue de CO_2 durant la période de stratification, on constate une absorption de CO_2 dans des incubations de sédiment superficiel in situ à 3,5 m. L'absorption de CO_2 est associée à un tapis épipélique d'algues bleu-vert qui semblent subir une photosynthèse anaérobie. Il semble donc y avoir simultanément photosynthèse et respiration anaérobies dans l'hypolimnion. On considère généralement les quotients respiratoires comme étant caractéristiques du métabolisme résultant de la nature du substrat biochimique oxydé par un organisme. Le quotient respiratoire de communautés mixtes d'organismes aérobies et anaérobies dépend davantage de la nature de l'agent oxydateur qui est réduit. L'écart entre le QR d'une communauté et celui anticipé (environs 0,85) reflète la proportion de métabolisme pour lequel l'oxygène n'est pas (encore) l'accepteur final des électrons. L'excès d'oxydation du carbone donne naissance à un pouvoir réducteur ambiant pouvant servir à la photosynthèse et à la chimiolithotrophie (anaérobies) bactériennes.

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PRELIMINARY measurements of O_2 deficits and CO_2 accumulation in Dunham Pond, Connecticut, suggested that benthic community respiratory quotients (RQ = CO_2 released/O_2 taken up) are variable and much greater than unity, particularly during the latter part of both summer and winter stratification (Rich 1975). An examination of data from five North American lake ecosystems also revealed variable and high RQs during periods of stratification (Rich and Devol 1978). While theoretical or "ideal" RQs based upon the aerobic respiration of known substrates are in the range of 0.5–1.0 (Ohle 1952; Teal and Kanwisher 1961; Kanwisher 1962; Boyd 1973), empirically determined benthic community RQs are frequently 3 or 4 times greater (up to 9 in Lake Wingra during winter stratification). These data cast doubt on the use of areal hypolimnetic oxygen deficits as a measure of benthic detrital metabolism, and imply that total lake bioenergetics have been traditionally underestimated.

Initially, the present study had two major objectives: (1) the investigation of the hydrologic, physical, and

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chemical stability and stratification at Dunham Pond to obtain estimates of mixing and diffusion in the water column, and (2) the simultaneous measurement of DIC (dissolved inorganic carbon) and dissolved O_2 changes in the hypolimnion and in situ incubations of the sediment surface to verify the suspected existence of elevated RQs. Data on the availability of underwater light and phytoplankton densities were collected to assist the interpretation of observed metabolism. The divergence of CO_2/O_2 metabolism results in the water column relative to the in situ sediment incubations was totally unexpected, and has inspired a subsequent analysis of ^{14}C uptake now in progress. A detailed analysis of the input–output and sediment–water fluxes of dissolved, redox-active substances constituting COD (chemical oxygen demand) is also in progress (R. W. Kortmann, personal communication, UConn, U-42). The ultimate objective of this research is to obtain a redefinition of lake ecosystem structure and function which will permit an integration of *all* material and energy transformations to be found beneath the surface of a lake.

Materials and Methods

Dunham Pond is a small (4.7 ha; 114 100 m^3), soft-water lake in the eastern Paleozoic highlands of Connecticut (Brooks and Deevey 1963). The drainage basin (44.8 ha) is second growth oak–hickory forest about 60 yr old. Only one house presently is within the catchment area. An intermittent upland stream from the east provides clear, relatively unmodified groundwater, while a red-maple wetland (4.0 ha) north of the lake discharges humic-stained water having high concentrations of dissolved organic carbon (DOC) and lowered concentrations of dissolved solids (Rich and Kowalczewski 1976). Annual contributions of water by the two streams are approximately equal. A preliminary study of the hydrology of the watershed and seepage into and out of the lake (Lee 1977) indicated that groundwater inputs are minor (R. W. Kortmann personal communication). Discharge from the upland stream and the outlet of the lake was measured by V-notch weirs. The total surface discharge (mean 1973–76 = 368 900 $m^3 \cdot yr^{-1}$) potentially replaced the water in the lake 3.2 times a year. Annual precipitation (mean = 108 cm) is rather evenly distributed through the year, but runoff is modified by freezing in the winter and evapo-transpiration in the growing season. Maximum discharge occurred in December (mean = 92 000 $m^3 \cdot yr^{-1}$), and little or no discharge occurred from May through September. Lake-volume based estimates of metabolism were corrected for water exchange in the lake only during winter stratification.

The lake has a shallow ($z_r = 1.8\%$), simple ($D_L = 1.1$) basin which has little wind fetch and is protected by surrounding hills. Its maximum depth is only 4.5 m ($\bar{z} = 2.4$ m), and a routine vertical series of five samples actually sampled more than half the length of the water column (sampler = 0.5 m) (Fig. 1). During stratification the most intense gradients occurred between the 2 and 3 m samples, and "hypolimnetic" metabolism was calculated as occurring below 2.5 m. The littoral zone is narrow, extending to about the 1.5-m contour, and occupied mostly by *Nuphar* and *Nymphaea*. At least half the shoreline is colonized by

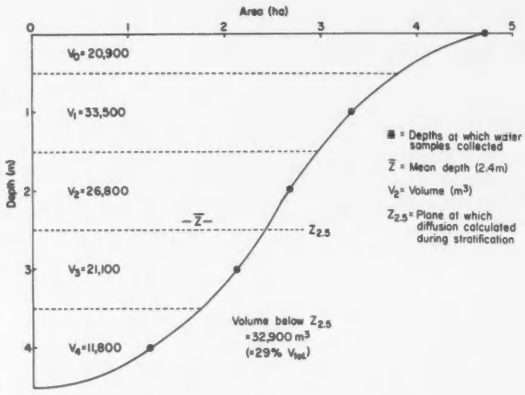

FIG. 1. Depth/area curve of Dunham Pond with sampling intervals and morphometric data used in lake-volume based calculations of metabolism.

Decadon. The benthic macroinvertebrate fauna consists largely of the midge *Chaoborus punctipennis* (85%) and the biting midge *Palpomyia tibialis* (10%) (Pallotti 1976).

Sampling occurred weekly or every two weeks from January 1973 to April 1976. Samples were obtained at the central station, the two inlet streams, and at the outlet. Light was measured with a cadmium sulfide cell (Rich and Wetzel 1969) and Secchi disk, temperature with a Whitney TC-5C thermistor (accuracy $\pm$ 0.1°C), and conductivity in the laboratory with a YSI Model 31 conductivity bridge and platinum electrode ($K = 0.1$). Dissolved oxygen was determined by the Winkler (azide) method, and dissolved CO_2 (DIC) with an MSA Model 202 (20″ cells) infrared-CO_2 analyzer. DOC was analyzed by the same equipment following thermal combustion. Chemical oxygen demand (COD) was estimated by injecting known volumes of water into BOD bottles already filled with well oxygenated distilled water, and then observing the amount of oxygen removed by the injected sample. The remaining oxygen content of the BOD bottle, adjusted for the displacement of oxygenated water by the anoxic sample, subtracted from the original oxygen content provided an estimate of oxygen equivalents of substrates which immediately gave up electrons in the presence of molecular oxygen and/or Winkler reagents (for more details, see Rich 1975). The phytoplankton samples were preserved with Lugol's solution, sedimented in Utermohl chambers, and counted with an inverted microscope.

Surface sediment samples were obtained at 3.5 m with a free-fall core sampler containing a clear plastic liner (4.6 cm ID; 50 cm length). Liners containing samples were placed in incubation racks which supported the sediment in the liners by adjustable internal plungers. Subsamples of the water overlying the sediments were removed with a syringe for determination of initial CO_2 and O_2 concentrations. Saran® wrap then was fastened tightly over the tops of the liners with rubber bands, air bubbles being carefully excluded, and the racks suspended in the lake so that the sediment–water interface of the sample chambers was at or near 3.5 m. Following incubation, generally 24 h, a syringe needle was punched through the Saran®, and water removed for determination of final CO_2 and O_2 concentrations. The

retention of CO_2 and O_2 by Saran® was found to be more dependable than that by solid acrylic tops carefully fitted to the liners (Rich 1970). Saran® was demonstrated to retain $H^{14}CO_3^-$ in the laboratory. Dissolved O_2 was determined by a semi-micro Winkler (azide) technique, and total DIC by IR analysis. The total volume of water incubated was measured by removal in a graduated syringe. A sample consisted of 4 replicate cores. Previous use of the method (Wetzel et al. 1972) indicated that metabolism begins to decline following an increment of 0.125 mM $CO_2 \cdot mL^{-1}$ in the chamber. Concentration changes that large were avoided by incubating large volumes of water (200–800 mL). The possible effects of reduced eddy diffusion in the incubation chambers is minimized in the case of Dunham Pond by the unusually low ambient diffusion coefficients (see below). Precautions to shade the cores from direct sunlight, and the relatively long incubation period, were used to minimize light shock (Van Baalen 1968).

The O_2 and CO_2 content of the lake was obtained as the sum of the products of concentration and volume at each depth sampled (Fig. 1) (Hutchinson 1957; Wetzel 1975). The O_2 deficits calculated for Dunham Pond do not represent an index of trophogenic productivity in the accepted sense. The lake does not meet the criteria recommended for the O_2 deficit method: the basin is too shallow, the water too highly colored, and the hypolimnion becomes anoxic soon after stratification (Hutchinson 1938, 1957; Wetzel 1975).

Results and Discussion

The mean light extinction coefficient (natural log) between the surface and 2.5 m in Dunham Pond was typically 2 or more. Low transparency is caused, in part, by high concentrations of dissolved and colloidal humic organic matter entering from the adjacent wetland. Dissolved organic carbon (DOC) ranges from 10 to 13 mg $C \cdot L^{-1}$ in the epilimnion throughout the growing season. The light extinction coefficient was generally greater than 2 above 1.5 m, and less than 2 below 1.5 m. In June and during fall overturn the extinction coefficients of both strata were briefly similar. The 1% light level (approximate compensation depth) was generally above 2.5 m, except for a brief period in May. (The 1% level also reached below 2.5 m in August 1974). Data from 1975 are presented for light intensities (Fig. 2) and phytoplankton densities (Fig. 6). The May transparency maximum occurred primarily above 1.5 m, despite a *Dinobryon* maximum. A diatom bloom occurred by June when light was being absorbed equally above and below 1.5 m. For the rest of the summer the light extinction coefficient above 1.5 m was 3 or greater, and phytoplankton densities above 2.5 m declined. Determination of the compensation depth as 1% light energy probably underestimated slightly the quanta at photosynthetically active wavelengths in Dunham Pond because of its brown color (Lewis 1975). However, the high densities of blue-green algae, both epipelic and planktonic, at 3 and 4 m could hardly be the product of conventional (aerobic) photosynthesis. The results of the in situ sediment incubations (see below) indicate anaerobic (low PQ) fixation of carbon at 3.5 m, and

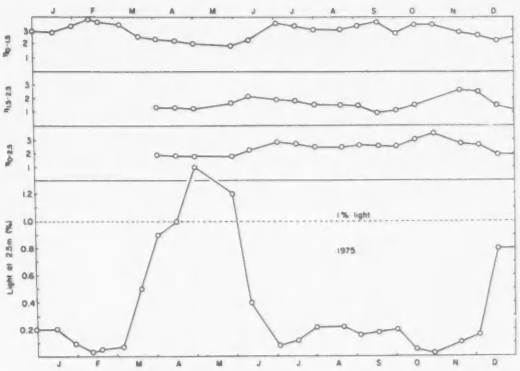

FIG. 2. Light data for 1975 at Dunham Pond.

preliminary results from subsequent ^{14}C uptake incubations in Dunham show important dark bottle fixation at the bottom during anoxia.

The typical summer thermal profile of Dunham Pond was equivalent to the metalimnion of a larger temperate lake, i.e. at temperature change of more than $1°C \cdot m^{-1}$ (Fig. 3). Thus, the use of the terms epilimnion and hypolimnion would not be correctly applied, although reference to these strata will be made for convenience. Much of the steepness of the thermal gradient was caused by high light extinction coefficients (i.e. the intensity of light absorption) of the water column. However, much heat also went through the bottom of the lake into the sediments which are thick relative to the shallow basin. Annual sediment heat budgets for 1973–75 (Table 2) were prepared by the method of Likens and Johnson (1969). Dunham lost an average of 29 kcal $m^2 \cdot d^{-1}$ to its sediments below 2.5 m during the sediment heating period, roughly similar to the average direct solar input below 2.5 m (37 kcal $m^2 \cdot d^{-1}$) and a third of the total heat stored there (91 kcal $m^2 \cdot d^{-1}$). Reversal of this heat flow stabilizes the water column in the winter by maintaining the bottom temperature near 4°C (Likens and Johnson 1969).

Eddy diffusion coefficients were determined during the heating seasons of 1973–75 by McEwen's technique (Hutchinson 1941, 1957; Idso and Cole 1973) (Fig. 4). Corrections were determined for solar and biogenic heating at 2.5 m, and for the thermal conduction of water (Bachman and Goldman 1965; Hesslein and Quay 1973) (Table 1). Heating rates tended to be slightly lower during the second half of the heating period, but this was ignored, as was geothermal heating and heat transferred by seiches (Dutton and Bryson 1962; Powell and Jassby 1974). Data collection was cut short in 1975 due to equipment failure.

Birgean and Forelian heat budgets did not compare very well (Table 2). The Birgean estimate, i.e. heat accumulation in the water column below 2.5 m (plus diffusion into the sediments), was less than the Forelian

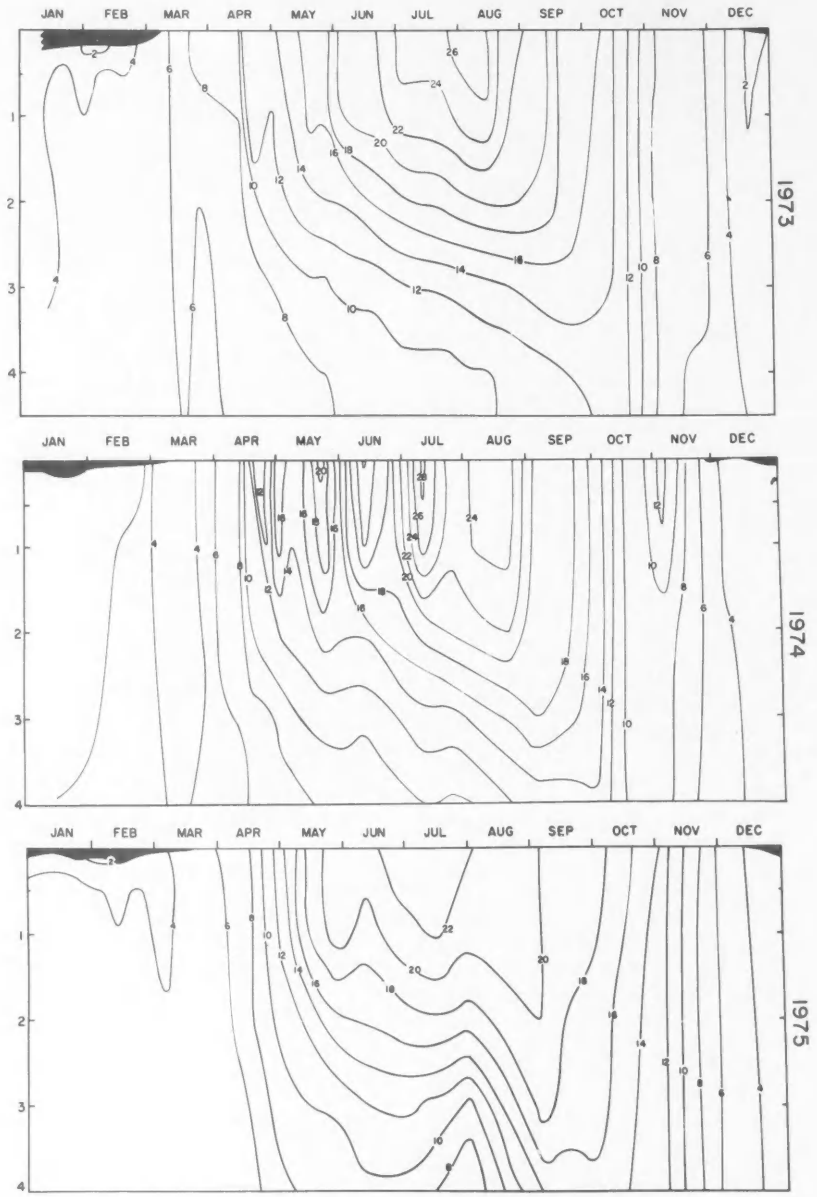

FIG. 3. Water temperatures 1973–75 at Dunham Pond (°C).

estimate, i.e. heat delivered through the 2.5 m plane, the two budgets are similar when eddy diffusion is omitted from the Forelian budget (but not omitting thermal conductivity). This similarity is probably fortuitous. Despite Hesslein and Quay's (1973) objections (based upon experiments in limnocorrals), McEwen's technique was verified convincingly by Stuiver's whole-lake results at Linsley Pond (1967) and more recently by Imboden and Emerson (1978). The lack of correspondence between heat delivered and heat stored beneath 2.5 m is the only potential evidence of exchange in the bathylimnion during the heating period. Other

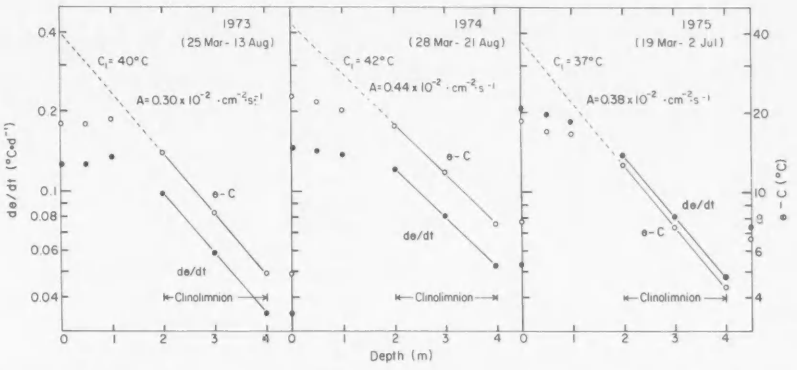

FIG. 4. McEwen analysis of heating in Dunham Pond 1973–75, and uncorrected coefficients of eddy diffusion.

possible explanations are heat losses generally considered negligible but potentially magnified by the small scale of Dunham Pond, such as back radiation (Walsh 1971) and diurnal effects (Miller and Reed 1975).

Vertical heterogeneity in specific conductance values during 1973–75 corresponds with, and confirms the integrity of, summer and winter stratification (Fig. 5). A detailed nutrient budget of Dunham Pond will provide further data on sediment–water fluxes of redox-active substances. Seasonal heterogeneity of conductance reflects the annual hydrologic cycle of the watershed and differential contributions of water from the upland stream and wetland. Precipitation is rather evenly distributed through the year in Connecticut, and the most important factor determining availability of groundwater at a site is the presence or absence of evapo-transpiratory demand by the predominantly deciduous terrestrial flora.

With the exception of the flora appearing in the "hypolimnion," the phytoplankton in Dunham Pond appears to be of an acidic–oligotrophic pond type (Round 1973; Hutchinson 1967). The first spring maximum was a desmid (*Cosmarium*), followed by a series of chrysophytes (*Ochromonas*, *Dinobryon*, and the diatoms *Melosira* and *Synedra*) (Fig. 6). A flagellate, *Peridinium* sp., bloomed just under the ice in February. However, under anoxic conditions and less than 1% surface light at 3 and 4 m a very different sequence appeared. During summer stratification two blue-green algae, *Lyngbya* sp. and *Oscillatoria* sp. dominated the anerobic hypolimnion. The *Lyngbya* sp. maximum started at 3 m in June and descended to 4 m for the rest of the summer. *Oscillatoria* sp. maintained a maximum at 3 m June–August. Neither alga appeared higher in the water column at any time. A brief maximum of *Microcystis* sp. appeared at 3 m in June, and *Anabaena* sp. appeared briefly at 1 m in July. An epipelic algal mat consisting of *Lyngbya* sp., *Oscillatoria* sp., and pamelloid *Aphanotheca* sp. appeared at 3.5 m during the latter part of both summer and winter stratification (Hornor 1977).

The segregation of the eukaryotic phytoplankton into the oxic epilimnion and the prokaryotic blue-greens into the anoxic hypolimnion is thought to be diagnostic in Dunham Pond for complete (anaerobic) and incomplete (anaerobic) electron transport (synthetic as well as oxidative, respectively). For the synthetic prokaryotes the presence of chemically reduced alternate (to H_2O) electron donors provides reducing power which contributes to the fixation (reduction) of CO_2. The alternate electron donors have their origin as alternate (to O_2) terminal electron acceptors in the anaerobic oxidation of organic matters to CO_2. The accumulation of alternate terminal electron acceptors reduced by

TABLE 1. Corrections of the McEwen eddy diffusion coefficients (1973–75) (8.64A·cm^{-2}·s^{-1} = K·m^{-2}·d^{-1}).

	K·m^{-2}·d^{-1}	% solar heating at 2.5 m	% biogenic heating at 2.5 m	Thermal conductivity	$K_{corr.}$
1973	26×10^{-3}	15%	0.2%	10×10^{-3}	12×10^{-3}
1974	38×10^{-3}	24%	0.1%	10×10^{-3}	19×10^{-3}
1975	33×10^{-3}	21%	0.2%	10×10^{-3}	16×10^{-3}
Mean	32×10^{-3}	20%	0.2%	—	16×10^{-3}

TABLE 2. Heat budgets below 2.5 m during the heating period of Dunham Pond (presented as daily heating rates, kcal·m^{-2}·d^{-1}).

	1973	1974	1975	Mean	
Birgean (below 2.5 m)					
Water column below 2.5 m	59	91	36	62	kcal·m^{-2}·d^{-1}
Sediment (diffusion)	26	32	29	29	
Total Birgean	84	123	65	91	
Forelian (below 2.5 m)					
Diffusion	68	112	31	70	kcal·m^{-2}·d^{-1}
Thermal conductivity	57	59	52	56	
Direct solar	22	54	37	37	
Biogenic	0.3	0.4	0.5	0.4	
Total Forelian	147	225	120	164	

anaerobic (prokaryotic) electron transport, whether in the sediments or the hypolimnion, represents reducing power conserved by the lake ecosystem. The potential importance and the theoretical implications of the phenomenon are described by Rich and Wetzel 1978.

LAKE-VOLUME BASED CO_2/O_2 METABOLISM

During the period between the onset of summer stratification and hypolimnetic anoxia (mean = 44 d), Dunham Pond was oligotrophic on the basis of its mean hypolimnetic O_2 deficit corrected for eddy diffusion (Fig. 7; Table 3) (Hutchinson 1957; Wetzel 1975). However, on the basis of hypolimnetic CO_2 accumulation (converting CO_2 to O_2 on the basis of the conventional RQ = 0.85) the lake was mesotrophic (0.030 mg O_2 (= mM $CO_2 \times 32/0.85$)·cm^{-2}·d^{-1}) during the period of CO_2 increase because the measured RQ averaged 2.2 (Table 3). In 1973 and 1974 Dunham Pond thermally stratified before the end of April (Fig. 3), and conductivity exceeded 70 μmhos·cm^{-2} at 4 m in September (Fig. 5). In 1975 thermal stratification was delayed about 2 wk, conductivity failed to reach 70 μmhos·cm^{-2} at 4 m in September, and the hypolimnetic RQ was correspondingly depressed (Table 3). During the first half of winter stratification (ice cover), O_2 uptake and CO_2 accumulation rates were almost identical on a molal basis (i.e. RQ = 1.0), and only about 20% less than summer rates before anoxia (Fig. 7; Table 4). During the second half of ice cover the concentration of O_2 near the sediments and O_2 uptake decreased, while the accumulation of CO_2 continued at rates similar to those found during the summer. Thus, as Ohle (1956) states, "... the accumulation of carbon dioxide is not limited in the hypolimnion because it is produced there."

Benthic anaerobic metabolism increases the apparent

TABLE 3. Hypolimnetic O_2 and CO_2 metabolism in Dunham Pond (A = until anoxia; B = during anoxia; Total = metabolic rate for the duration of CO_2 accumulation; T + D = total metabolic rate corrected for eddy diffusion). Rates determined by linear regression analysis when $n \geq 5$.

		1973	1974	1975	Mean	Mean + diffusion
Oxygen metabolism (mM O_2·m^{-2}·d^{-1})	A	5.8	5.0	5.2	5.3[a]	7.0
		$r = 0.99$	0.96	0.94		
	B	0.1	0.0	0.0	0.0	1.3
	Total	1.8	1.6	2.8	2.1	3.6
	T + D	2.7	3.7	4.4	3.6	—
Carbon metabolism (mM C·m^{-2}·d^{-1})	A	6.5	6.4	4.7	5.9	7.9
		r = —	0.96	0.93		
	B	4.3	3.9	4.3	4.2	8.2
		$r = 0.99$	0.96	0.95		
	Total	4.6	4.5	4.3	4.5	7.8
		$r = 0.99$	0.98	0.98		
	T + D	7.2	9.7	6.4	7.8[b]	—
Respiratory quotient ($RQ = CO_2/O_2$)	A	1.1	1.3	0.9	1.1	1.1
	B	—	—	—	—	6.3
	Total	2.6	2.8	1.5	2.1	2.2
	T + D	2.7	2.6	1.5	2.2	—
	Period (days)	121	132	105	119	
	Eddy diffusion ($\times 10^{-3}$·m^{-2}·d^{-1})	12	19	15	15	

[a] = 0.017 mg O_2·cm^{-2}·d^{-1} (less than 0.025 = oligotrophic).
[b] = 0.029 mg O_2 (equiv.)·cm^{-2}·d^{-1} (0.025 to 0.055 = mesotrophic).

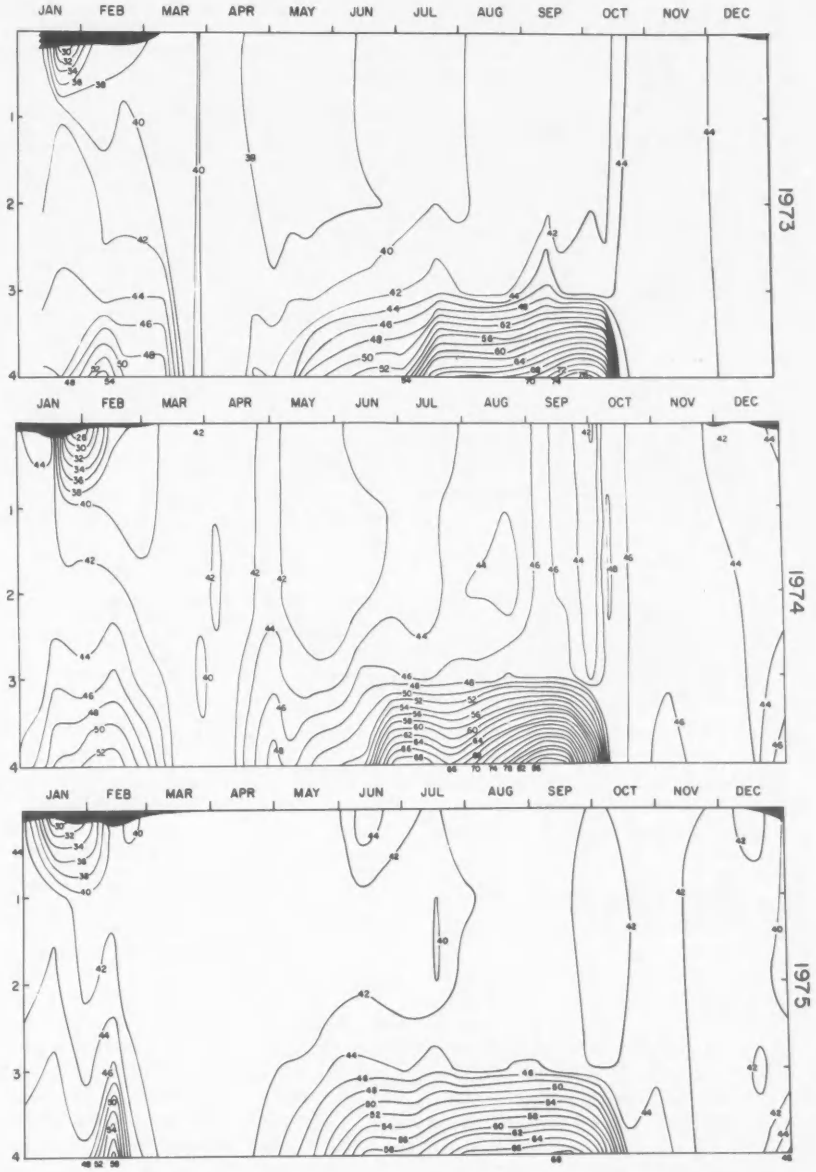

FIG. 5. Conductivity data for 1973–75 at Dunham Pond (μmhos·cm^{-2}, 25°C).

benthic community respiratory quotient (RQ = CO_2 released/O_2 taken up). A respiratory quotient for a eukaryotic organism is generally characteristic for the biochemical substrate being oxidized by intermediary metabolism. (Subsequent biosyntheses may modify the net RQ). Carbohydrates are the most oxidized substrates commonly available for metabolism, and the RQs of eukaryotic organisms respiring carbohydrates cannot be significantly greater than one under steady-state. The respiratory quotient of a mixed community of aerobes and anaerobes, however, can be greater than one because of the nature of the oxidizing agents being reduced by oxidative phosphorylation and/or fermentation. The degree to which the actual RQ of

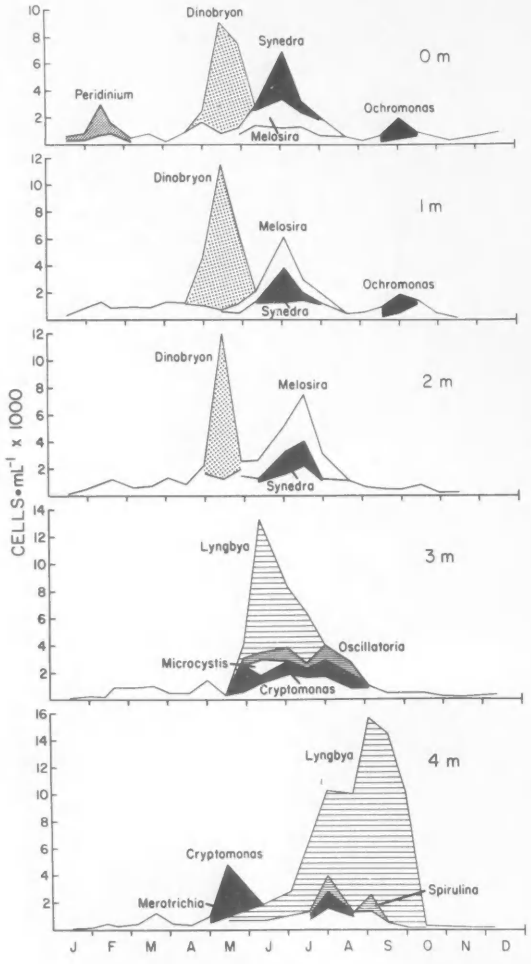

FIG. 6. Annual phytoplankton densities for 1975 in Dunham Pond (cells $mL^{-1} \times 10^3$).

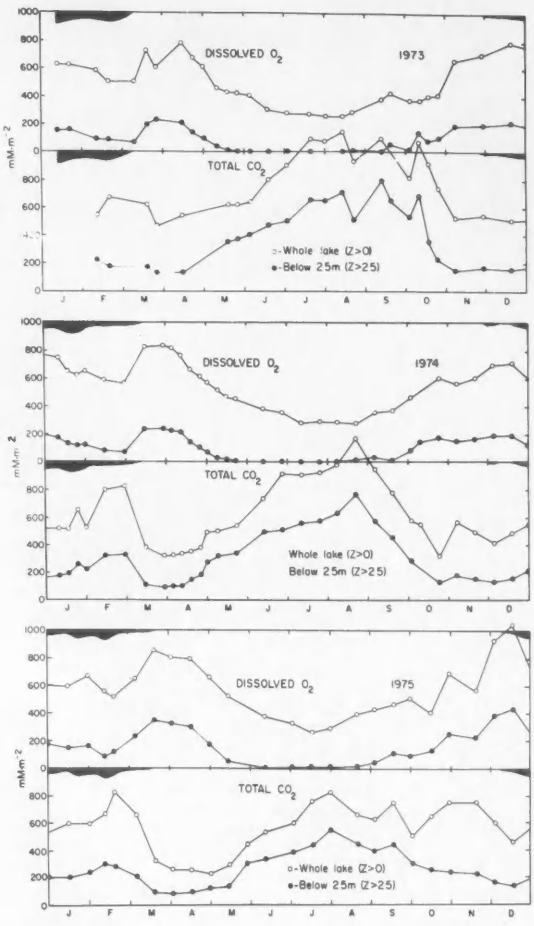

FIG. 7. O_2 and CO_2 content of Dunham Pond 1973–75 ($mM \cdot m^{-2}$).

prokaryotes is greater than the expected RQ (about 0.85) is an indication of the amount of metabolism for which oxygen is not the terminal electron acceptor. Important alternate terminal electron acceptors (oxidizing agents) in lakes include ferric iron, nitrate, and sulfate. The presence of these alternate electron acceptors permits prokaryotes to oxidize organic matter in the absence of molecular oxygen, thereby releasing CO_2 without the concomitant uptake of O_2.

The disposition of the residual reducing power not used to reduce oxygen to water can be estimated from CO_2/O_2 metabolism and then tested as ambient COD. For example, during the height of summer stratification (mid-June through July) in 1973 CO_2 accumulated below 2.5 m at an estimated 4.1 mM $CO_2 \cdot m^{-2} \cdot d^{-1}$ and diffusion progressed at the rate of 3.2 mM $CO_2 \cdot m^{-2} \cdot d^{-1}$, for a gross rate of release of 7.3 mM $CO_2 \cdot m^{-2} \cdot d^{-1}$. The stratum below 2.5 m was anoxic, and O_2 uptake was limited to diffusion, i.e. an estimated 1.3 mM $O_2 \cdot m^{-2} \cdot d^{-1}$. During the same period the intensity of COD below 2.5 m increased at the rate of 3.9 mM O_2 equiv. $\cdot m^{-2} \cdot d^{-1}$. A calculation of COD equivalents from the release of CO_2 in excess of O_2 uptake can be made, $7.3 - 1.3 = 6$ mM COD O_2 equiv. $\cdot m^{-2} \cdot d^{-1}$, which is one and a half times greater (6/3.9) than observed. This result suggests a significant diffusion rate for COD and/or an important donation of electrons to anaerobic photosynthesis (or chemosynthesis) at the aerobic/anaerobic interface in the water column. I have insufficient data to do a statistical treatment of these estimates (particularly difficult with the diffusion coefficient) but find the basic proportions

TABLE 4. Winter O_2 and CO_2 metabolism in Dunham Pond (A = first half of winter stratification; B = second half; Total = metabolic rate for the duration of ice cover; T + E = total metabolic rate corrected for exchange of water). Rates determined by regression analysis when $n \geq 5$. During 1975 and 1976 a thaw occurred about midway in stratification which caused most of the observed exchange and necessitated new regression lines. Regression statistics in parentheses were obtained by removing the effects of the thaws, i.e. removing the change in intercepts of the regression lines representing the first and second parts of stratification.

		1974	1975	1976	Mean	Mean + exchange
Oxygen metabolism	A	4.4	3.9	3.6	4.0	6.7
(mM $O_2 \cdot m^{-2} \cdot d^{-1}$)	B	2.0	7.5	5.0	4.8	6.3
	Total	3.2	(4.7)	(4.1)	4.0	6.1
		$r = 0.93$	(0.97)	(0.99)		
	T + E	5.4	6.4	6.6	6.1	—
Carbon metabolism	A	2.8	4.2	3.5	3.5	6.1
(mM $C \cdot m^{-2} \cdot d^{-1}$)	B	7.2	10.3	9.6	5.3	9.0
	Total	5.0	(6.0)	(5.7)	5.6	8.8
		$r = 0.83$	(0.96)	(0.93)		
	T + E	8.3	8.1	10.0	8.8	—
Respiratory quotient	A	0.6	1.1	1.0	0.9	0.9
($RQ = CO_2/O_2$)	B	3.6	1.4	1.9	2.3	1.4
	Total	1.6	1.3	1.4	1.4	1.4
	T + E	1.5	1.3	1.5	1.4	—
	Period (days)	71	63	69	68	
	Exchange	1.5	1.1	1.5	1.4	

compelling. The observed accumulation of reduced terminal electron acceptors (COD) was half (3.9/7.3) of benthic metabolism as measured by CO_2 release and 3× (3.9/1.3) greater than the metabolic rate indicated by O_2 diffusion.

Collateral investigations are in progress at Dunham which pursue the origin and constituents of COD. Hornor (1977) determined that COD in the top 2 cm of sediment at Dunham Pond was generally greater than 50 mM COD O_2 equiv. $\cdot m^{-2}$ throughout the year and that it increased to over 100 mM COD O_2 equiv. $\cdot m^{-2}$ 8 cm into the sediments. Kortmann (1977) found an almost 10-fold increase of ferrous iron in the water column between May and September (up to 60 μg-at Fe^{++} L^{-1} in the water immediately overlying the sediments, and up to 90 μg-at Fe^{++} L^{-1} in the interstitial water of the sediments). His data for mid-June through August 1976, represent an increase in COD intensity of about 1 mM COD O_2 equiv. $\cdot m^{-2} \cdot d^{-1}$, suggesting a rapid turnover of iron and involvement of other redox-active species in COD.

Relative rates of CO_2 and O_2 metabolism changed as stratification progressed, and their trajectories suggest potential limitations of either electron acceptors or organic matter (electron donors). Carbon metabolism increased slightly while the RQ increased sharply at Dunham Pond, indicating the mobilization of alternate electron acceptors (Fig. 8). Lawrence Lake, Michigan, had a similar response, while Mirror Lake, New Hampshire, showed both increasing CO_2 and O_2 metabolism during a modest increase in RQ (Rich and Devol 1978). Both Dunham and Lawrence lakes appear electron acceptor limited relative to Mirror Lake which appears organic matter limited.

IN SITU SEDIMENT INCUBATIONS

Light data and volume-based CO_2/O_2 metabolism to the contrary, the in situ sediment surface incubations were very significantly "photosynthetic" (i.e. CO_2 uptake and O_2 production) during both summer and winter stratification (Table 5). These data, obtained from November 1973 to September 1976, are variable, especially during the latter part of summer stratification, but the annual pattern is very distinct. CO_2 production, O_2 uptake (respiration), and RQs near unity occurred during periods of circulation and during the last vestiges (September–October) of "metalimnion erosion." CO_2 uptake occurred during periods of low oxygen concentrations, and PQ (O_2/CO_2) values were depressed to 0.2–0.3, indicating bacterial photosynthesis. However, small concentrations of dissolved O_2 actually appeared in the cores, indicating the temporary exhaustion of ambient COD as an electron donor by the blue-green mat. This strong autotrophic element in the sediment samples, taken and incubated at 3.5 m, suggests that the sediments and water beneath the 3.5 contour and the epipelic mat were more actively heterotrophic.

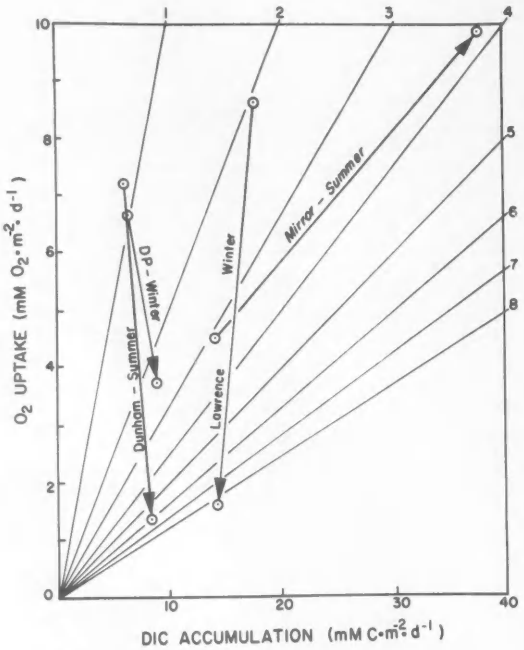

FIG. 8. Trajectories of mean CO_2 accumulation and mean O_2 uptake between the first and last parts of summer and winter stratification at Dunham Pond (1973–75). Data for Lawrence and Mirror Lakes from Rich and Devol (1978). The numbered lines radiating from the origin represent respiratory quotients.

Thus, the unusually low PQ values tend to balance the high RQ values (CO_2/O_2) during the same period. This raises the likelihood of an anaerobic hypolimnetic carbon cycle in which alternate terminal electron acceptors produced by high RQ (mostly anaerobic) respiration become electron donors to low PQ (mostly anaerobic) photosynthesis.

The opposite situation occurred at Lawrence Lake, a hard-water lake in Michigan, for which both in situ and lake-volume CO_2/O_2 data also exist (Rich and Wetzel 1972; Rich and Devol 1978). During summer stratification more CO_2 leaves the benthic sediment surface (in situ incubations = 18.9 mM $CO_2 \cdot m^{-2} \cdot d^{-1}$), than accumulates in the hypolimnion (accumulation plus diffusion = 5.4 mM $CO_2 \cdot m^{-2} \cdot d^{-1}$). Despite the greater depth of Lawrence Lake, the 1% light level just reached the bathylimnion (Rich and Devol 1978). In both Dunham and Lawrence the bottom of the bathylimnion appears to be oxidizing carbon without reducing oxygen, and the top of the bathylimnion appears to be reducing carbon without oxidizing water. Verduin (1971) reported the same phenomenon in a sewage treatment lagoon, and postulated a photosynthetic process which does not produce O_2. More recently, bacterial production (dark uptake of $^{14}CO_2$) at Solar Lake, Elat, was found to be much higher than could be explained by chemorganotrophy (Cohen et al. 1977). High benthic RQs suggest that carbon oxidation proceeds in this situation, that reducing power will not be found on organic matter, and that chemolithotrophy would be involved. Further, benthic respiration having high RQ values is a likely source of the reducing power subsidizing light utilization efficiencies in the metalimnion of Lake Kinneret (Dubinsky and Berman 1976).

Conclusion

The intensity of light/heat absorption in the water column and the heat capacity of the sediments of Dunham Pond combine to create a stable thermal gradient, either direct or inverse, throughout most of the year. Within the physical structure is an electron gradient (reducing power) originating from the high RQ oxidation of benthic detritus. At the other end of the gradient are two electron "predators": oxidative and photophosphorylation (i.e. oxygen and light). The residual reducing power remaining from incomplete oxidative electron transport during respiration by prokaryotes in

TABLE 5. In situ sediment incubation data from Dunham Pond (recalculated from Hornor 1977).

	n	Carbon metabolism (mM $C \cdot m^{-2} \cdot d^{-1}$)	Oxygen metabolism (mM $O_2 \cdot m^{-2} \cdot d^{-1}$)	RQ or PQ
Fall overturn				
mean	15	4.9	4.8	1.0 RQ
95% conf. int.		±1.4	±1.3	±0.3
Winter stratification	2	−5.7	−1.5	0.3 PQ
Spring overturn	25	3.7	4.6	0.8 RQ
95% conf. int.		±1.3	±0.9	±0.4
Summer stratification				
Heating period	11	−9.1	−2.0	0.2 PQ
95% conf. int.		±2.9	±0.7	±0.9
Metalimnion erosion	10	7.7	5.7	1.3 RQ
95% conf. int.		±10.8	±3.7	±1.5

the hypolimnion becomes available to chemolithotrophs and chemorganotrophs in the presence of molecular oxygen. When oxygen is not competing with photosynthesis for electrons, low intensity and high entropy radiation is sufficient to reduce CO_2, although the energy transformation is not equivalent to oxic photosynthesis to the extent that the electron donor subsidizes the process. Thus, the creation of ambient reducing power by high benthic RQ values represents an important bioenergetic factor, the fundamental nature of which has been obscured by the conventional use of benthic O_2 uptake data and "ideal" RQ values (e.g. 0.85). These conventions simultaneously underestimate benthic metabolism and sweep an important category of energy under a conceptual rug.

This conclusion may appear obvious and even trivial. Its full application, however, appears absent in a number of instances. Ohle (1952; 1956) deals with benthic CO_2 production in excess of O_2 uptake by observing that half of organic matter undergoing anaerobic metabolism is oxidized to CO_2 and that the balance represents the annual increment of organic sediment (less soluble or volatile products such as methane). The 1956 paper does not explicitly refer to the reducing power represented by alternate electron acceptors following anaerobic metabolism, and the 1952 paper presents the energy as calories (based on glucose) without being explicit about its potential fates. Nauwerck (1963) suggested bacterial sources for the excess productivity of zooplankton over phytoplankton in Lake Erken. Dark CO_2 uptake created by a benthic electron gradient derived in part from allochthonous and littoral sources of organic matter provides a likely origin. Edmondson (1966) found that hypolimnetic O_2 deficits did not increase with algal productivity during the latter stages of the eutrophication of Lake Washington, and presented a case for lessened zooplankton predation in the water column to account for the lack of increased benthic metabolism. Phytoplankton–zooplankton interactions appear to be a reasonable explanation when benthic respiration, estimated by O_2 uptake, is only one-third of primary production (Edmondson et al. 1956). However, while Lake Washington has a long period of circulation, its annual mean benthic RQ is likely to be between mesotrophic Lawrence Lake (0.8–3) and eutrophic Lake Wingra (?–9) rather than 0.85 as assumed (Rich and Devol 1978). Thus, the benthic metabolism of carbon is likely to be equal to or greater than phytoplankton productivity. In Lake Mikolajskie (Poland) inputs of energy to the profundal zone through sedimentation ($188\ kcal \cdot m^{-2}$) are much greater than energy output ($76\ kcal \cdot m^{-2}$) calculated from O_2 deficits (Kajak et al. 1975). Similarly, Provini's (1975) inability to classify lakes by O_2 deficits is not surprising, and might be resolved by examining empirical RQ values. The benthic RQ of Vorderer Finstertaler See might offer an explanation for its high CO_2 uptake (Tilzer 1972). The unusual proportions of autochthonous production and sediment accumulation in Lawrence Lake relative to other lakes, observed by Saunders (1976), may be the difference between a complete and deficient definition of ecosystem energetics as Wetzel et al. (1972) asserted. With the exception of certain bacteriologically inclined limnologists, particularly Sorokin (e.g. 1964, 1965, etc.), there is little explicit in limnological literature to call attention to the importance of benthic respiration. The evident lack of curiosity about the discoveries of high RQ values and low PQ values in shallow marine systems 15 yr ago (e.g. Park et al. 1958; Odum et al. 1963; Copeland and Jones 1965; etc.) (and in freshwater by Wood and Verduin in 1972) is puzzling, as is the preference of oxygen-based, indirect estimates of carbon metabolism when the CO_2–pH change technique (Beyers and Odum 1959; Beyers et al. 1963) is available and possibly even more convenient. However, the concepts involved are embedded in the more general evolution of the trophic–dynamic paradigm which systematically underestimated the role of detritus (Rich and Wetzel 1978).

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Fish Viruses: A Double-Stranded RNA Icosahedral Virus from a North American Cyprinid

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A previously unreported virus disease of cultured golden shiners (*Notemigonus crysoleucas*) is described. The condition is called golden shiner virus (GSV) disease. The virus is icosahedral, measures approximately 70 nm, is ether and heat resistant, stable at pH 3, 7, and 10, and appears to have a double stranded RNA core. It was not neutralized by infectious pancreatic necrosis virus specific antiserum. GSV causes syncytial like cytopathic effect in fathead minnow cells but not brown bullhead, rainbow trout gonad (RTG-2) or channel catfish ovary cells. Severe mortalities in golden shiner culture ponds do not appear to be associated with GSV infections.

Key words: fish viruses, RNA virus, fish pathology, fish cell cultures, Cyprinidae, golden shiner

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Les auteurs décrivent une maladie à virus non signalée à ce jour chez des chattes de l'est (*Notemigonus crysoleucas*) d'élevage. On lui donne le nom de maladie à virus de la chatte de l'est (GSV). Le virus est icosaèdre, mesure approximativement 70 nm, est résistant à l'éther et à la chaleur, stable aux pH 3, 7 et 10, et semble avoir un centre d'ARN à deux brins. Il n'est pas neutralisé par l'antisérum particulier au virus de la nécrose pancréatique infectieuse. Le GSV entraîne une prolifération du noyau dans les cellules du tête-de-boule mais non dans celles de la barbotte brune, non plus que dans les cellules des gonades de truite brune (RTG-2) ou d'ovaires de barbue de rivière. Les fortes mortalités de chattes de l'est dans les étangs d'élevage ne semblent pas liées aux attaques du GSV.

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SINCE the development of fish virology in the middle 1950s, viruses have been isolated from a variety of fish families including salmonids, cyprinids, esocids, ictalurids, and others (Wolf 1976). The more devastating viruses of fish infect those species which are economically important in aquaculture, such as trout and catfish. Spring viremia of carp (Fijan et al. 1971) is a very important disease of cultured carp *Cyprinus carpio* in Europe. Bait minnows are the primary cyprinids cultured in the United States, principally the fathead minnows, *Pimephales promelas*, and the golden shiner, *Notemigonus crysoleucas*.

During late summer of 1977 when water temperatures ranged from 27 to 32°C, we examined several groups of golden shiners, from two widely separated geographical regions, for viral diseases. Necropsy demonstrated a lack of parasitic or bacterial pathogens. A filterable agent was isolated in fathead minnow cells (FHM) (Gravell and Malsberger 1965), which at the present time appears to be a previously undescribed virus. Affected golden shiners were listless, swimming near the surface, diving when disturbed, but soon returning to the surface. Dying fish sometimes swam in a spiral. Macroscopic petechial hemorrhages occurred in the cornea and the dorsal musculature resulting in a reddish back; petechiae were present on ventral body surfaces, in visceral fat, and in the intestinal mucosa. Relatively low rates of mortality were associated with the infection.

Methods and Materials

Initially three groups of frozen golden shiners from culture ponds were examined, while a fourth group of moribund shiners was obtained from ponds at a fisheries research facility. The viscera of all groups of fish were triturated in a mortar and pestle prior to dilution to 1:10 with Hanks balanced salt solution (HBSS). The homogenate was centri-

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fuged at 1500 rpm for 10 min and the supernatant passed through a 0.45-μm Millipore filter. Tissue culture flasks (25 cm^2) with monolayers of FHM cells and Eagles minimum essential medium (EMEM) supplemented with 10% fetal calf serum, penicillin (100 IU/mL), streptomycin (100 μg/mL), and Gentomycin (50 μg/mL), were inoculated with 0.5 mL of filtrate from each sample of suspect fish.

After a filterable agent was suspected, media from infected FHM cells was serially transfered through three consecutive cell cultures. Biochemical and biophysical characteristics of the agent were determined using either disposable microtiter (Linbro) or multiwell 2.2 cm^2 tissue culture plates (Falcon). After seeding with sufficient FHM cells to form a monolayer, wells were immediately inoculated in triplicate with material to be assayed, unless otherwise stated. Inoculated cultures were incubated at 30°C in plastic tubes purged with sufficient CO_2 to maintain pH 7.2–7.4 and covered with plastic wrap. Unless otherwise stated, cultures were examined for CPE daily for 5 d and then fixed with 10% formalin, stained with 1% crystal violet and read again with a dissecting scope. Tissue culture infectious dose-50% end point ($TCID_{50}$) were calculated by the Reed and Muench (1938) method.

Two separate groups of 6–8 cm (7.3 cm average) golden shiners were injected intraperitoneally (IP) with 0.1 mL of a virus suspect dilution containing $10^{4.5}$ $TCID_{50}$/0.1 mL. Control fish were similarly injected with EMEM without virus and all fish were held in 30-L aquaria. Initially five fish were injected with 3rd passage GSV and held at 21–22°C for 3 wk. Moribund fish were removed and the visceral organs assayed for virus. The second group of experimentally infected golden shiners consisted of 20 fish injected IP with 0.1 mL of the 6th passage virus with the same titer as previously used and held at 28°C. At 3-d intervals after injection the liver, kidney, and spleen of three test and three control fish were assayed for virus and the pooled organ homogenates were titrated in FHM cell cultures.

Heat lability of the virus was determined by preparing a 1:10 dilution (HBSS) of media from infected cells and incubated at 50°C for 30 min. Nonheated control and heated test material were titrated in FHM cells. Ether sensitivity was determined according to the method of Andrews and Horstman (1948). A 10^{-1} dilution of media from infected cells was made in 20% ethyl ether and incubated at 5°C for 18 h along with a nonether treated control and then titrated.

Sensitivity of the agent to hydrogen ion concentration was tested according to Mascoli and Burrell (1965). A 1:10 dilution of media from infected cells was made in Tris buffer adjusted to pH 3, pH 7, and pH 10. Samples were incubated at 25°C and each sample was titrated at 30-min intervals for 120 min.

The effect of 5-iodo-2 deoxyuridine (IDU) on the virus was determined by preparing a 10^{-4} M solution of IDU in EMEM which was used to prepare serial 10-fold dilutions of the virus. FHM cells in media with IDU were seeded into microtiter wells and triplicate wells were inoculated with 0.1 mL of the diluted virus. Control cells were inoculated with similar virus dilutions using media without IDU.

RNAse activity on the virus was determined by the method of Gomatos and Tamm (1963). A solution of RNAse containing 0.1 μg/mL was used to prepare a 1:10 dilution of media from infected cells and incubated at 30°C at pH 7 for 20 min. An RNAse free control was maintained and both solutions were then titrated. The effect of DNAse on the virus was determined by the method of Lin and Maes (1967). A 1:10 dilution of media from infected cells was made in a solution containing 5 mcg/mL of DNAse and another 1:10 dilution in HBSS. Both were incubated at 37°C for 30 min at pH 6.5–7 and then titrated.

FHM cultures on glass cover slips in Leighton tubes were infected with the infectious agent and after 24 h the cell cultures were placed in acid–alcohol fixative and stained with acridine orange (0.01%) with citric acid buffer. The cover slip cultures were mounted on slides and examined with a fluorescent microscope.

FHM cells which demonstrated cellular damage (cytopathic effect -CPE) were prepared for transmission electron microscopy. The infected cells were scraped from a flask and fixed in 1% osmium tetroxide. After being embedded in epoxy, ultrathin sections were made with a glass knife and stained with uranyl acetate and lead citrate.

Cell cultures of rainbow trout gonad (RTG-2) (Wolf et al. 1960), channel catfish ovary (CCO) (Bowser 1978), and brown bullhead (BB) (R. G. Malsberger and C. P. Cerini, Lehigh University) were inoculated with culture media from infected FHM cells. The RTG-2 cultures were incubated at 20°C while the CCO and BB cells were incubated at 30°C and observed for CPE for 2 wk.

A cell associated virus (CAV) and a cell free virus (CFV) replication curve was made in FHM cells. Thirty-six drained FHM monolayers in 35 × 10 mm culture plates were inoculated with 0.1 mL of virus containing 3.6×10^6 $TCID_{50}$/0.1 mL, producing a multiplicity of approximately 2 $TCID_{50}$ per cell. Virus was allowed to adsorb at 30°C for 1 h and then each cell sheet was washed with HBSS three times, 2 mL of EMEM-10 added, and the cultures were returned to 30°C. At 2 h intervals thereafter one plate was removed for virus assay. Media was aspirated from the cell sheet and stored in a culture tube at 5°C until titered. The cells were then scraped from the surface and resuspended in 2 mL of culture media. The cells were immediately disrupted by sonication with a Bronson sonifier (Model 140) for 3 s at 20 Khz at 60 w and stored for a minimum of 12 h at 5°C until titered, however, most samples were immediately processed.

Polyvalent anti-infectious pancreatic necrosis virus (IPNV) rabbit serum, and control serum were obtained from the National Fisheries Center (Leetown), Kearneysville, W. Va. Serial two-fold dilutions of each sera were made in HBSS to 1:640. Equal amounts (0.3 mL) of serum dilution and virus with approximately 100 $TCID_{50}$/0.1 mL were combined and incubated at 30°C for 30 min. Each serum dilution-virus mixture was then innoculated into triplicate FHM monolayers in microtiter plates and incubated at 30°C for 4 d at which time the plates were read for virus CPE.

Results and Discussion

An apparently, previously undescribed, filterable agent was isolated in FHM cell cultures inoculated with filtered visceral homogenates from golden shiners. Small, circular, focal areas of cell destruction appeared in all inoculated cultures in 18–24 h. However, after 48 h the focal cytopathic effect (CPE) in two cultures were overgrown with apparently healthy cells, and subsequent blind passages of these cultures failed to elicit CPE in inoculated cultures. The third culture continued to

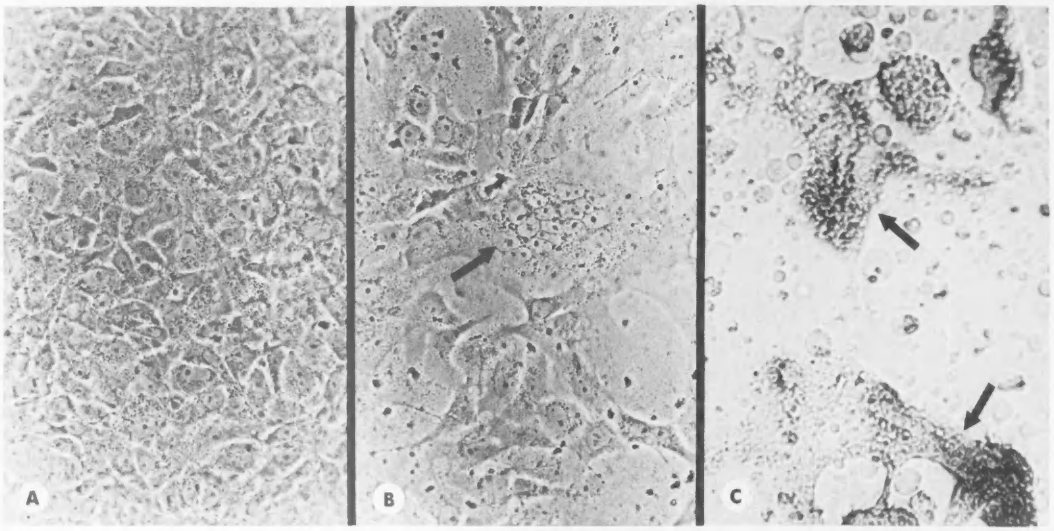

FIG. 1. Fathead minnow cells (FHM) infected with golden shiner virus (76×). (A) Non infected control cells. (B) Focal CPE 18 h after inoculation. Note multinucleated and vaculated area (arrow). (C) Massive CPE 24 h after inoculation. Note the large accumulation of cellular material within a membrane (arrow).

deteriorate involving the entire cell sheet. Media from this culture was filtered and transferred to healthy FHM cells where CPE progressed as before. A second passage was made with identical results followed by serial cell culture passes. CPE in FHM cells is characterized by the formation of focal syncytia with multiple nuclei and cellular vacuolation 16–18 h post inoculation at 30°C (Fig. 1). The CPE progresses rapidly and at 24 h the cell sheet begins to contract and large, rounded particles of cellular debris separate from the culture surface (Fig. 1). Often some cell sheet regeneration will follow the development of CPE. The media contains unattached round, vacuolated cells of various sizes and the cell sheets are much more sparsely populated than controls.

Three of five golden shiners injected with 3rd passage virus suspect material died 7–13 d later. A filterable agent from the viscera of these fish was isolated in FHM cells producing identical CPE as in the primary isolation. No fish died in the second experimental transmission study although virus was isolated from the viscera of the virus injected fish. Visceral organ titers were $10^{5.69}$ $TCID_{50}$/0.1 g at 3 d, $10^{5.17}$ $TCID_{50}$/0.1 g at 6 d, $10^{1.02}$ $TCID_{50}$/0.1 g at 10 d, and $10^{1.02}$ $TCID_{50}$/0.1 g at 13 and 17 d. No virus was isolated from either control group. Rivers postulates were partially fulfilled for the virus; it was successfully transmitted from culture to host and reisolated, but the clinical signs of the disease did not develop. This suggests that the agent has very low pathogenicity, although virus exists in the host, for over 2 wk.

The virus is not sensitive to 20% ether (Table 1), which indicates the absence of a lipoprotein envelope. Also the virus is not heat labile as the titers of control and test solutions were very similar in the heat and ether treatments (Table 1). Hydrogen ion concentration did not affect the viability of the virus (Fig. 2). Titers were very similar at time 0 and after 120 min exposure at pH 3, pH 7, and pH 10. There were no differences in titers between samples exposed to the different pHs.

Acridine orange stained infected FHM cultures had yellowish green cytoplasmic inclusions, not present in the control cultures, which is indicative of formation of a double stranded nucleic acid. RNAse treatment, which degrades single stranded RNA, did not reduce

TABLE 1. Stability of golden shiner virus after various physical and chemical treatments.

Treatment	Virus titer ($TCID_{50}$/0.1 mL)	
	Treated virus	Untreated virus
RNAse	1.5×10^{5}	2.4×10^{5}
DNAse	2.1×10^{5}	3.2×10^{5}
IDU[a]	3.2×10^{6}	3.2×10^{6}
Ether[b]	1.5×10^{6}	6.8×10^{6}
pH 3[c]	6.6×10^{4}	3.2×10^{4}
pH 7[c]	3.2×10^{4}	6.6×10^{4}
pH 10[c]	6.6×10^{4}	3.2×10^{4}
50°C for 30 min	6.8×10^{6}	6.8×10^{6}

[a]5-iodo-2 deoxyuridine.
[b]20% ether at 5°C for 18 h.
[c]25°C for 120 min.

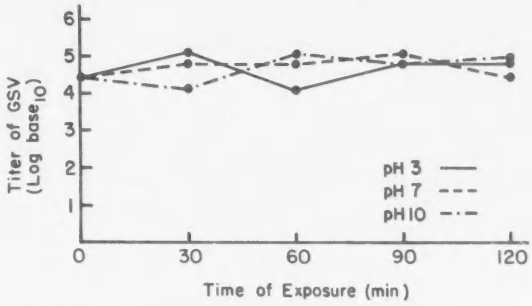

FIG. 2. Sensitivity of golden shiner virus to pH.

infectivity (Gamatos and Tamm 1963). DNAse and IDU also failed to affect the infectivity, thus it is concluded that presumptively the virus consists of a double stranded RNA nucleic acid.

Virus infected FHM cells had cytoplasmic paracrystalline arrays of icosahedral viruses (Fig. 3). These virions had no envelope, as suggested by the ether sensitivity test. The virion measured 70 nm in diameter, agreeing with differential filtration during preliminary studies in which the virus was passed by a 100 nm membrane, but was retained by a 50 nm membrane. The paracrystalline array was surrounded by a distinct area of hyalinization. Few virions were seen disassociated from the crystalline arrays.

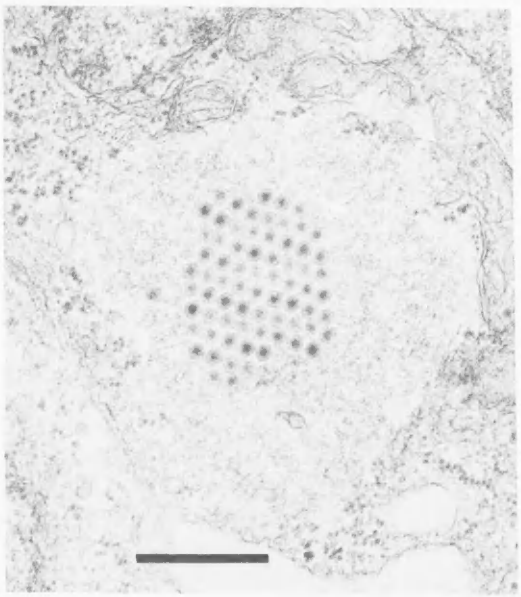

FIG. 3. Golden shiner virus in FHM cell. A paracrystalline array of icosahedral virions lies in the cytoplasm and is surrounded by hyalinization. Scale 0.5 μm.

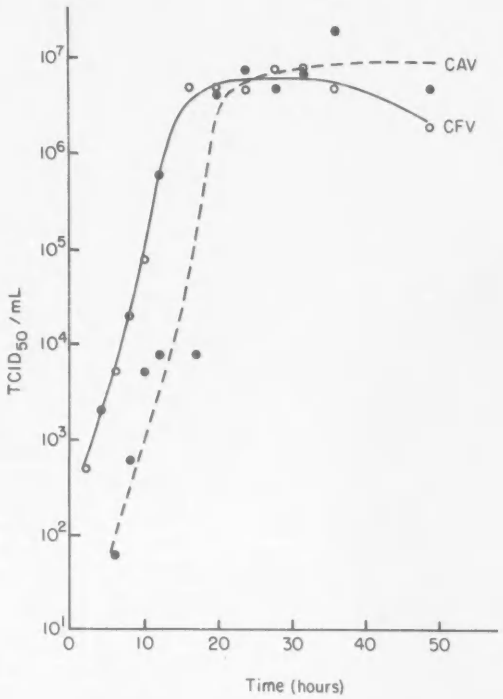

FIG. 4. Growth curve of cell free and cell associated GSV in FHM cells inoculated with a multiplicity of approximately 2 $TCID_{50}$ per cell.

The lag phase in the growth curve was 2–4 h. Cell associated and cell free virus curves were similar in slope, but the cell free curve was approximately one log (base 10) higher than the cell associated curve (Fig. 4) at all steps during the initial 20 h postinfection. At 30°C cell free and cell associated levels of virus reached maximum titers at approximately 24 h postinfection after which the CFV began to decline and the CAV remained relatively stable.

Neutralization tests with polyvalent IPNV specific antiserum failed to affect infectivity of the virus from golden shiners. Polyvalent IPNV antiserum and control serum had titers of less than 1:5 against approximately 100 $TCID_{50}$ per 0.1 mL of golden shiner virus.

We propose the name "golden shiner virus" (GSV) for the etiology of "golden shiner virus disease" (GSVD). Preliminary investigations indicate that GSV is very similar to the Reoviridae (Table 2) (Wilner 1969; Fenner 1974; and Anon. 1975). Presumptively GSV has a double stranded RNA genome, but whether or not it is 10 separate paired segments as the Reoviridae or two paired segments as in IPNV (Dobos 1976) is not known. It is an icosahedral virus measuring approximately 70 nm, has no envelope and replicates cytoplasmically. Other Reoviridae similarities are its

TABLE 2. Characteristics of golden shiner virus (GSV), infectious pancreatic necrosis virus (IPNV), and the Reoviridae.

Characteristic	GSV	IPNV[a]	Reoviridae[b]
Tissue culture susceptibility	FHM	RTG-2, FHM and others	Varies
Size	70 nm	60–70 nm	70 nm
Symmetry	Icosahedral	Icosahedral	Icosahedral
Capsid	Unknown	Single	Double
Ether	Not sensitive	Not sensitive	Not sensitive
pH 3	Not sensitive	Not sensitive	Not sensitive
Heat	Not sensitive	Not sensitive	Not sensitive
Nucleic acid	Probably double stranded RNA, segmentation unknown	Double stranded RNA, 2-segments	Double stranded RNA, 10 segments
IPN antiserum neutralization	Negative	Positive	—

[a]Coons et al. (1974); Kelly and Lob (1975).
[b]Wilner (1969); Dobos (1976).

lack of ether, heat and low pH sensitivity, however whether or not it has a double capsid, as the Reoviridae, is not known. GSV is also similar morphologically, biophysically, and biochemically to infectious pancreatic necrosis virus (IPNV) of salmonids (Table 2) (Cohen et al. 1973; and Kelly and Loh 1975). However GSVs capability of replicating at 30°C, its lack of RTG-2 infectivity, and the inability of polyvalent IPN antiserum to neutralize GSV, distinctly separates GSV from IPNV. Furthermore no other fish viruses causes a similar CPE in fish cells. More complete characterization is needed before proper classification can be proposed.

The effect of GSV on the minnow industry is presently unknown. However, there appear to have been no massive mortalities in golden shiner culture ponds as a result of GSV, even on farms from which these initial isolations were made. Our laboratory experiments bear out the probable low pathogenic nature of GSV. Under normal golden shiner culture, the disease appears to chronically occur in crowded populations during summer, resulting in rather low mortalities among larger shiners. However since the initial isolations of GSV, a virus was isolated from golden shiners in a minnow dealers tank which compares favorably to GSV. Approximately 75% of 12 000 shiners died, but whether or not other compounding factors were involved could not be ascertained.

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New Information on the Longevity of Pacific Ocean Perch (*Sebastes alutus*)

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BEAMISH, R. J. 1979. New information on the longevity of Pacific ocean perch (*Sebastes alutus*). J. Fish. Res. Board Can. 36: 1395–1400.

A comparison of ages determined from surfaces of otoliths and from sections of otoliths showed that ages were similar up to a section age of 22–24 yr. The growth pattern observed on thin sections of otoliths and from broken and burned otoliths from the region of the nucleus was interpreted to indicate that Pacific ocean perch probably live much longer than previously thought. Because the criteria used to identify annuli from otoliths sections appeared valid for younger fish, there was no justification to reject the application of these criteria for estimating ages of older fish and thus no reason to reject the possibility that Pacific ocean perch might live to be older than 70 yr. Ages determined from sections of otoliths from other rockfish species found off the west coast of Canada such as *Sebastes flavidus* and *Sebastes brevispinis* have ranged from 30 to 60 yr indicating that many species of rockfish may live longer than previously thought.

Key words: age determination, otolith, maximum age, age determination errors, Pacific ocean perch, otolith sections

BEAMISH, R. J. 1979. New information on the longevity of Pacific ocean perch (*Sebastes alutus*). J. Fish. Res. Board Can. 36: 1395–1400.

Une comparaison des âges déterminés sur des surfaces et sur des coupes d'otolithes démontrent une similarité d'âges jusqu'à 22–24 ans déterminés sur des coupes. Les modalités de croissance observée sur de minces coupes d'otolithes et sur des otolithes cassés et brulés dans la région du noyau indiquent, d'après notre interprétation, que le sébaste du Pacifique a une durée de vie probablement beaucoup plus longue qu'on ne l'avait cru précédemment. Les critères utilisés pour identifier les annuli de coupes d'otolithes semblant valides pour les jeunes poissons, il n'est pas justifiable de rejeter ces critères lorsqu'il s'agit d'estimer l'âge de plus vieux poissons. Il n'y a donc pas de raison de rejeter la possibilité que le sébaste du Pacifique puisse dépasser 70 ans. Les âges déterminés à partir de coupes d'otolithes d'autres espèces de sébastes rencontrés au large de la côte occidentale du Canada, tels *Sebastes flavidus* et *Sebastes brevispinis*, s'étalent de 30 à 60 ans, donnant à croire que plusieurs espèces de sébastes peuvent vivre plus longtemps qu'on ne l'avait cru précédemment.

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PACIFIC ocean perch (*Sebastes alutus*) for many years has been the single most important species in the groundfish fishery off the west coast of Canada. As a consequence of heavy foreign fishing early in this decade, many stocks were over exploited and attempts are now being made to rebuild these stocks. During a routine comparison of age readings from the otolith surface and cross sections (through the nucleus) of the same otolith it became apparent that some of the largest and oldest fish in the sample were much older when annuli were determined from the cross sections. Because of the importance of this species and the probable application of these results to other rockfish species, investigators should be aware of the importance of examining sections of otoliths when estimating the age of rockfish.

Materials and methods — Pacific ocean perch were obtained in large numbers as part of an exploratory fishing cruise off the west coast of Vancouver Island. The location of sets, a description of gear and composition of catches have been summarized in Sigmund et al. (1979). One of the samples used for this study was obtained when the catch was being processed on shore and consisted of a total sample of the first 411 fish from a bulk lot of a mixture of Pacific ocean perch from all sets. A second sample of 10 individuals was selected from a sample of older fish (by surface readings) collected in 1978 from Moresby Gully

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TABLE 1. Surface age determinations for Pacific ocean perch from otoliths collected in this study and from Moresby Gully in 1974[a].

Age (years)	West coast Vancouver Island 1978				Moresby Gully 1974			
	No. of fish			% of total	No. of fish			% of total
	♂	♀	♂+♀		♂	♀	♂+♀	
3					2	0	2	—
4					17	25	42	2.1
5					25	19	54	2.7
6					16	17	33	1.7
7		1	1	0.2	21	19	40	2.0
8	1	1	2	0.5	41	38	79	4.0
9	2	3	5	1.2	46	40	86	4.4
10	6	12	18	4.4	61	46	107	5.4
11	16	22	38	9.3	71	51	122	6.2
12	27	28	55	13.5	72	53	125	6.6
13	41	30	71	17.4	73	59	132	6.7
14	36	17	53	13.0	50	16	66	3.4
15	33	18	51	12.5	50	21	71	3.6
16	23	14	37	9.1	57	29	86	4.4
17	15	13	28	6.9	91	35	126	6.4
18	7	4	11	2.7	98	56	154	7.8
19	3	2	5	1.2	98	63	161	8.2
20	0	2	2	0.5	94	67	161	8.2
21	2	3	5	1.2	80	39	119	6.0
22	2	5	7	1.7	55	37	92	4.7
23	1	5	6	1.5	32	29	61	3.1
24	1	2	3	0.7	16	19	35	1.8
25		4	4	1.0	4	6	10	0.1
26		4	4	1.0	2	1	3	—
27		1	1	0.2				
28		1	1	0.2				
Total	216	192	408		1172	795	1967	

[a]Unpublished data courtesy Mr S. J. Westrheim combined from 200, 235, 270, 310, and 340 m. Moresby Gully is located in southern Hecate Strait, approximately 52°30′N, 130°20′W.

in southern Hecate Strait. The surface age of the otolith was estimated according to the method described by Westrheim (1973) by a technician thoroughly experienced with the application of this technique to Pacific ocean perch. A subsample of three individuals from each age-class as determined from surface readings was selected from the west coast Vancouver Island sample for sectioning. Otoliths which were most easily aged from the surface were selected. Otoliths were mounted, sectioned, and examined according to the procedures described by Beamish (1979). A large sample of Pacific ocean perch collected from the relatively unexploited stocks in Moresby Gully in 1974 has been included in the study for comparison with the relatively heavily exploited stocks off Vancouver Island.

If annuli could not be easily identified from the thin section, it was possible to heat the epoxy containing the remaining piece of otolith on a hot plate and soften the epoxy so that the otolith face could be removed and burned in a flame from an alcohol burner. The burned surface was then either ground off until growth zones were clearly visible when painted with cedarwood oil, or painted with cedarwood oil without grinding similar to the procedure described by Chugunova (1959). The breaking, polishing, burning, and cedarwood oil technique was an important alternative approach to the thin section method. Thin sections were preferred in this study because the identification and counting of the narrow zones in otoliths from older fish was facilitated by the use of phase contrast microscopy and higher magnifications.

Results and discussion — Ages determined from the otolith surface (Table 1) ranged from 7 to 28 yr with a modal age of 17 yr. The translucent zone was considered to be the annulus when examining sections of otoliths. In transmitted light this zone appears as a light band that is narrower than the opaque zone in younger fish but becomes equal in width or even wider than the opaque zone for older fish (Fig. 1). Annuli from otolith sections could be distinguished most easily in the area on either side of the central groove or sulcus acusticus, the groove on the interior surface of the otolith that contains the auditory nerve. While an alternating pattern of translucent and opaque zones was visible on all sections, annuli in most sections were not as easily identifiable as from the otoliths shown in Fig. 1. Zones are more difficult to identify from the early period of otolith growth than from the portion of the otolith that corresponds to growth in later years. The difficulty in-

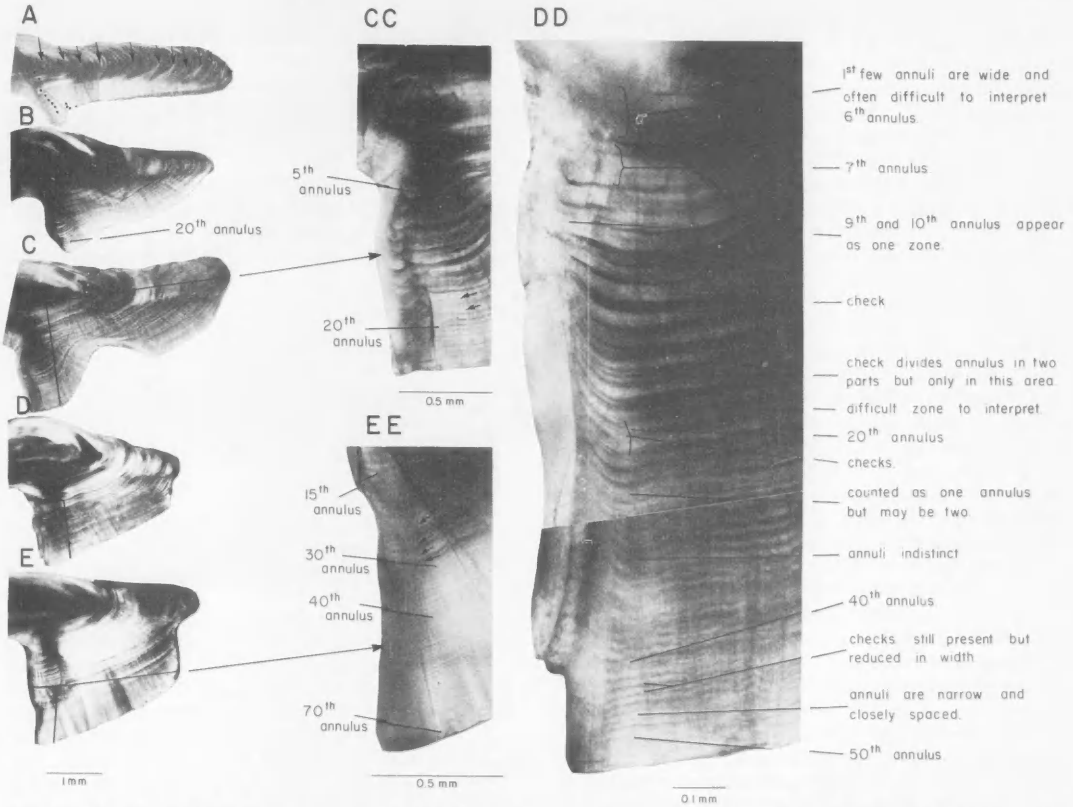

FIG. 1. Sections of Pacific ocean perch otoliths. The narrow annuli representing the older years are not visible from the photography of otoliths D and E but are visible on the enlarged portion in sections DD and EE. Annuli were more distinct when viewed with a microscope than they appear from the photographs. A, ♂ 35 cm, surface age 12, section age 12, arrows indicate position of annuli identified from surface dots indicate annuli identified in the area of the central groove; B, ♂ 45 cm, surface age 24, section age 24, arrows indicate position of discontinuity of an annulus; C, ♀ 46 cm, surface age 24, section age 28; D, ♂ 41 cm, surface age 26, section age 54; E, ♂ 42 cm, surface age 27, section age 72, arrows indicate prominent checks; CC, DD, EE enlarged portion of the central grooves area of otolith C, D, E, respectively, the arrows show prominent checks or areas of discontinuities. Otoliths A, C were from fish from off the west coast of Vancouver Island and B, D, E from fish from Moresby Gully. Section DD enlarged portion of section D showing the relative size of annuli as they became more narrow and closer spaced. Also shown are some of the difficulties encountered when attempting to interpret the growth pattern.

terpreting annuli for the younger years results in part from a lack of prominence of the translucent zone in the vicinity of the interior groove and partly because the first few translucent zones are wide and may contain several checks. Checks are also present in annuli that correspond to older years and such checks can produce a thin opaque zone within a translucent zone. The thickness of the check relative to adjacent opaque zones is usually sufficient to distinguish the check from an annulus. Counts of annuli from sections of very old fish may not be constant simply because of the mechanics of counting the large number of narrow annuli that sometimes are obscure.

On many otolith sections it was not possible to show that all the annuli identified from the exterior surface were the same annuli that were counted on the edge of the central groove. However, the otolith sections in Fig. 1 were very good and it is apparent from many of these sections that the translucent zone visible on the surface was the same zone counted in the vicinity of the central groove. In Fig. 1A independent determinations of surface and section annuli resulted in an identical count of 12. The position of the first 2 annuli is unclear in the area of the central groove and the 7th and 8th annuli appear combined in the photograph but could be separated when viewed with a microscope. Note that the 7th and 8th annuli that would be visible from the surface were also closer together. The otolith

in Fig. 1B was also aged as 24 yr from sections and surface readings. Otoliths C, D, E all were aged older from sections than from surface examinations. It is very important to note that not only do these Otoliths contain more lines (or annuli), they are thicker than otoliths from the younger fish. There also is a progressive decrease in the thickness of each zone found on the edge of the interior groove. For example the width of the combined 15th, 25th, and 50th opaque and translucent zones in otoliths C, D, E range from 0.052 to 0.033, 0.033 to 0.020, and 0.010 to 0.008 mm, respectively. Otoliths that had more than 25 to 30 annuli had an accummulation of the very narrow zones in addition to the thicker zones that were characteristic of the younger otoliths (Fig. 1, CC, DD, EE). Some annuli on most sections were not as easily identified as others because of checks or because they appeared to become confluent with adjacent annuli (Fig. 1, CC, DD, EE). Annuli seldom could be identified as a continuous zone in all parts of a section and counts of annuli in areas of discontinuities (Fig. 1B) should be avoided. Despite the difficulty in obtaining precise readings and despite the difficulty in identifying the position of a particular zone in all parts of the section it was apparent that a pattern existed and it remained to be shown that the pattern could be interpreted to allow an estimate of the age of the fish.

It would be extremely difficult to verify that all zones identified as annuli from the otolith section did form once a year since no method presently is available to successfully tag and release live Pacific ocean perch. However it is possible to compare section ages with surface ages and for the period of years that the age determination method has been validated by Westrheim (1973) for surface readings it is possible to validate indirectly the interpretations applied to otolith sections.

When this comparison was made (Fig. 2) it was found that up to a section age of 22–24 yr the deviation of the mean surface age from each corresponding section age showed no trend and the values were scattered on either side of the zero deviation line. After section age 22 there was a distinct trend of increasing deviation with increasing section age indicating that surface age determination reached a maximum while annuli continued to be identifiable on the interior surface. Also no very old (>20 yr) ages were obtained by the section method for any of the fish aged less than 20 yr by the surface method. Section ages of younger fish could be considered to be similar to surface ages. It is apparent from Fig. 1 that the zones that form in later years form a very regular pattern. If the zones that form in later years are not annuli it would be necessary to postulate that the growth pattern of the otolith of each of the older fish is different from the younger fish and that a change in the pattern of zone formation occurred after an age of about 22–24 yr. This appears unlikely since after a section age of about 5–10 yr the growth zones (interpreted as annuli) from otolith sections exhibit a similar and constant pattern

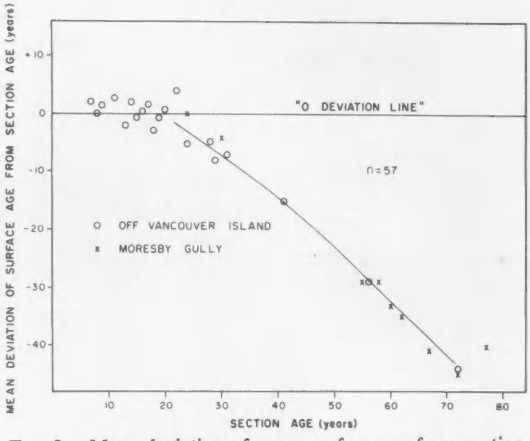

FIG. 2. Mean deviation of mean surface age from section age.

of decreasing thickness. Anomalies in pattern formation are not apparent for fish older than 15–20 yr (Fig. 1). When the technique of breaking an otolith in half and burning the polished face was used, it was possible to examine annuli in more detail than was possible for most thin sections. Using this technique it could be shown that in most cases while the width of the annulus changed with time, the annulus did not bifurcate or change in some way that might result in a zone that formed annually being considered to be two or more annuli.

Older male Pacific ocean perch aged by the section method were smaller than females of similar age but neither males nor females showed a trend towards increased length with increased section age (Table 2). While the sample size in this study is small and the validity of extrapolating conclusions about growth from grouped samples is unknown it is possible that after a certain length, fish may increase in age with little or no increases in length. That is, the maximum length might be obtained before the maximum age is obtained. According to Beverton and Holt (1960) the concept that natural life span extends far beyond the time when maximum size is reached is rare or unknown in fishes. It may be argued that some growth in length perhaps measured in very small units is occurring but certainly growth is minimal and for all practical purposes growth in length appears negligible. Also, if the ages determined from sections are correct, the possibility that decreases in length occur should not be ignored.

The oldest age recorded for Pacific ocean perch is 30 yr (Westrheim 1970) but it is acknowledged that the species may be much older since annuli are difficult to identify from the surface of older fish as they tend to "roll over" the edge of the otolith and can only be viewed by tilting the otolith (S. J. Westrheim, Pacific Biological Station, Nanaimo, personal communication).

TABLE 2. Length and sex of Pacific ocean perch older than 25 yr as determined from the section method. Samples from Moresby Gully are indicated by (M).

Section age (yr)	Surface age (yr)	Fork length (cm)
Males		
30 (M)	27	44
55 (M)	26	41
58 (M)	29	41
60 (M)	28	42
62 (M)	27	43
67 (M)	26	42
72 (M)	27	42
77 (M)	27	41
Females		
28	22	45
28	23	46
28	25	46
30 (M)	25	45
31	24	46
36 (M)	26	46
41	26	47
56	27	48
72	28	45

It may be difficult to believe that the maximum age could be more than double this figure. Yet we can accept that some fishes do get to be very old. Sturgeon (*Acipenser fulvescens*) have been reported to reach a maximum age of over 80 yr (Probst and Cooper 1954) and one report records a sturgeon as being 152 yr old (Anon. 1954). The spiny dogfish (*Squalus acanthias*) is thought to attain ages of over 60 yr (Wood et al. 1979) and Power (1978) has suggested that the life span of lake trout (*Salvelinus namaycush*) and lake whitefish (*Coregonus clupeaformis*) may exceed 50 yr. It also has been reported that ages determined for the rockfish or redfish (*Sebastes mentella*) from the exposed surface of an otolith cracked transversely through the nucleus were much older than previously thought (Sandeman 1961). Sandeman (Biological Station, St. John's, Newfoundland, personal communication) reports that in virgin stocks, a significant portion of the stock ranged in age from 40 to 50 yr and some of the older fish showed signs of senility. The oldest redfish found in their studies was aged as approximately 83 yr. Thus there is evidence that some fishes can live to ages we consider to be old (an obvious relationship to our own life span).

In this study an examination of the pattern of growth visible on the interior of the otolith has shown that surface and section age determinations agree up to a section age of 22–24 yr. The annulus continues to be visible from the otolith section resulting in an accummulation of annuli of progressively narrowing width and an increase in the lateral thickness of the otolith. Thus Pacific ocean perch appears to be simply another fish that can live to be very old.

It would appear from the small sample examined in this study that any fish aged 22–24 yr or older from the otolith surface should also be aged using otolith sections. Otolith readers familiar with Pacific ocean perch otoliths often do not attempt to age fish past an age of 20 yr. Therefore a more cautious approach should be to section any otolith aged to be 20 yr or more from surface readings. In the heavily exploited stocks found off Vancouver Island, 8% of the sample was aged to be 20 yr or older (Table 1). However in the less heavily exploited or relatively unexploited stocks found in Moresby Gully, 24% of the fish were age 20 or older (Table 1). An analysis of the consequences of having some very old fish in a stock of Pacific ocean perch with respect to the population dynamics of the stock and strategies involved in the rehabilitation of overexploited stocks are beyond the scope of this report and are currently being studied as part of the program studying groundfish stocks found off the west coast of Canada.

The procedure of sectioning all otoliths that were aged as 20 yr or older from surface age determinations was applied to other species of rockfish found off the west coast of Canada. In many cases it was found that ages determined from sections were older than the age determinations from the otolith surface. For example surface age determinations of otoliths from *Sebastes aleutianus* that ranged from 24 to 28 yr, ranged from 30 to 40 yr when determined from sections of otoliths. Otolith sections from *Sebastes brevispinis* and *Sebastes flavidus* have been aged as high as 60 and 56 yr, respectively, and similar observations have been found for *Sebastes paucispinis*, *Sebastes borealis*, *Sebastes reedi*, *Sebastes diploproa*, and *Sebastes proriger*.

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Temperature Influence on Basal Activity and Induction of Mixed Function Oxygenase Activity in *Fundulus heteroclitus*[1]

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STEGEMAN, J. J. 1979. Temperature influence on basal activity and induction of mixed function oxygenase activity in *Fundulus heteroclitus*. J. Fish. Res. Board Can. 36: 1400–1405.

Treatment of *Fundulus heteroclitus* acclimated to 6.5°C with benzo(*a*)pyrene did not elicit any change in the levels of hepatic microsomal NADH- or NADPH-cytochrome c reductase activity, nor in the levels of cytochrome P-450 or its catalytic activities. However, the same treatment of fish at 16 5°C resulted in a marked induction of benzo(*a*)pyrene hydroxylase and NADPH-cytochrome c reductase. Cytochrome P-450 content was also higher in the warm, treated fish and the Soret maximum of reduced, CO-treated microsomes was shifted to the violet. Levels of aminopyrine demethylase and NADH-cytochrome c reductase activities did not show a significant treatment effect. At neither temperature could treated and control fish be distinguished on the basis of in vitro inhibition of benzo(*a*)pyrene hydroxylase activity by 7,8-benzoflavone. Levels of NADPH-cytochrome c reductase and benzo(*a*)pyrene hydroxylase activities were greater in control *Fundulus* acclimated to 6.5°C than to 16.5°C, when normalized to microsomal protein, but not when based on body weight. The results indicate that habitat temperature alone may not affect the capacity for initial hydrocarbon metabolism in fish, but that it can strongly influence the induction of cytochrome P-450.

Key words: temperature, cytochrome P-450, hydrocarbon metabolism, mixed-function oxygenase, *Fundulus heteroclitus*

STEGEMAN, J. J. 1979. Temperature influence on basal activity and induction of mixed function oxygenase activity in *Fundulus heteroclitus*. J. Fish. Res. Board Can. 36: 1400–1405.

Le traitement au benzo(*a*)pyrène de *Fundulus heteroclitus* acclimatés à 6,5°C ne produit aucun changement de niveau d'activité de la NADH- ou de la NADPH-cytochrome c réductase microsomique hépatique, non plus que des niveaux de cytochrome P-450 ou de ses activités catalytiques. Cependant, le même traitement appliqué à des poissons à 16,5°C déclenche une activité prononcée de la benzo(*a*)pyrène hydroxylase et de la NADPH-cytochrome c oxydase. La teneur en cytochrome P-450 est aussi plus élevée chez les poissons traités à la température plus élevée, et le maximum Soret des microsomes traités au CO et réduits glisse vers le violet. Les niveaux d'activité de l'aminopyrine déméthylase et de la NADH-cytochrome c réductase ne montrent pas d'effet notable à la suite du traitement. A ni l'une ni l'autre température peut-on distinguer les poissons traités des témoins en se basant sur l'inhibition in vitro de l'activité de la benzo(*a*)pyrène hydroxylase par le 7,8-benzoflavone. Les niveaux d'activité de la NADPH-cytochrome c réductase et de la benzo(*a*)pyrène hydroxylase sont plus élevés chez des *Fundulus* témoins acclimatés à 6,5°C que chez ceux acclimatés à 16,5°C, lorsque normalisés par

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rapport aux protéines microsomiques, mais non lorsque basés sur le poids corporel. Ces résultats indiquent que la température ambiante seule peut ne pas affecter la capacité de métabolisme initial des hydrocarbures chez les poissons, mais qu'elle peut influencer fortement l'induction du cytochrome P-450.

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CYTOCHROME P-450-mediated metabolism of aromatic hydrocarbons in marine fish appears to function efficiently in promoting the disposal of these compounds (Lee et al. 1972) and in activating them to cytotoxic and mutagenic products (Stegeman 1977; Stegeman et al. 1979). Experimental induction of cytochrome P-450 in fish is known to increase the rates of such metabolism, whether measured in vitro or in vivo (Statham et al. 1978). Such induction could have biological consequences and also, if occurring in the environment, serve to indicate effects of certain environmental chemicals (Payne 1976; Stegeman 1978). Changes in other environmental conditions might, however, mitigate the action of inducers and affect the rates of foreign compound metabolism.

Temperature is known to regulate a variety of metabolic and biochemical functions in poikilotherms (Fry 1958; Hazel and Prosser 1974). It seems quite likely that temperature differences should also influence fish mixed-function oxygenases. A previous study has indicated that a change in habitat temperature can alter basal levels of cytochrome P-450 dependent mixed-function oxygenase activity in fish (Dewaide 1970). However, it was unclear from that report which microsomal electron transport components or functions might have been responsible for the differences associated with temperature. Moreover, there is no information on how temperature might influence induction of mixed-function oxygenases in fish. The present report, therefore, describes the levels and induction of hepatic mixed-function oxygenase activities, and other aspects of microsomal electron transport systems, in *Fundulus heteroclitus* acclimated to two different temperatures.

Materials and methods — Chemicals — Benzo[*a*]pyrene (Gold Label), aminopyrine and 7,8-benzoflavone were obtained from Aldrich Chemical Company (Milwaukee, WI). NADP, NADPH, NADH, glucose-6-phosphate, glucose-6-phosphate dehydrogenase, horse heart cytochrome c, Tris, and HEPES were all obtained from Sigma Chemical Company (St. Louis, MO).

Animals — *Fundulus heteroclitus*, 5–15 g, were obtained locally (Popponessett Bay, Mashpee, MA) and maintained in 70-L aquaria equipped with flowing seawater at 16.5 ± 0.5°C for 2 mo prior to use. Prior to and during experiments all animals were fed a diet of chopped smelt, ab libitum, every 3 d. Three weeks prior to experimentation one group of animals was acclimated to water from the same source, but cooled to 6.5 ± 0.5°C. After the acclimation period, one group of animals at each temperature was injected intraperitoneally with a corn oil suspension of benzo[*a*]pyrene, using a dose equivalent to 10 mg/kg. Another group at each temperature was injected with fresh corn oil only. Animals were returned to their respective tanks, with controls upstream and separated by mesh. Fish from each of the two experimental and two control groups were pair-sampled at 48 and again at 96 h after injection. Fish were not undergoing gonadal development and thus not separated according to sex.

Fish were sacrificed by decapitation and whole livers were immediately excised and placed in ice cold buffer. Tissues were homogenized as before (Stegeman and Binder 1979) in 10 volumes of 0.065 mol/L phosphate buffer, pH 7.0 containing 3 μmol/L $MgCl_2$ and 1.15% KCl, using a Potter–Elvejhem tissue grinder. Microsomal fractions, prepared as previously described (Stegeman and Binder 1979), were resuspended in 0.1 mol/L phosphate buffer pH 7.3 to a concentration of 2–3 mg protein/mL.

Enzyme assays — NADPH-cytochrome c reductase was assayed at 25°C by a modification of the method of Phillips and Langdon (1962), with a reaction mixture containing 0.175 mmol/L NADPH and 80 μmol/L horse heart cytochrome c in 0.2 mol/L potassium phosphate buffer, pH 7.7. Microsomal NADH-cytochrome c reductase was assayed using the conditions for NADPH-cytochrome c reductase, with 0.25 mmol/L NADH replacing the NADPH.

Benzo(*a*)pyrene hydroxylase was assayed in a 0.5 mL reaction mixture containing 0.7–1.0 mg microsomal protein and an artificial NADPH-generating system (Stegeman 1978) in 0.1 mol/L Tris-HCl, final pH 7.3. The reaction was initiated by addition of benzo(*a*)pyrene to 60 μmol/L, with or without 7,8-benzoflavone, and incubated 15 min at 25°C. Reactions were stopped and hydroxylated product extracted and assayed fluorometrically in a procedure similar to that of Nebert and Gelboin (1968) using authentic 3-OH benzo(*a*)pyrene as a reference standard.

Aminopyrine demethylase was assayed as before (Stegeman and Binder 1979) in 0.166 mol/L HEPES buffer, final pH 7.6. Formaldehyde generated was determined according to Nash (1953) as modified by Cochin and Axelrod (1959). Formaldehyde standards were incubated and treated as samples and blanks for both benzo(*a*)pyrene hydroxylase and aminopyrine demethylase consisted of reactions carried out without NADPH.

Microsomes were diluted to 0.27–0.34 mg protein per mL in 0.1 mol/L phosphate buffer, pH 7.3, and reduced, CO-bound cytochrome P-450 was analyzed optically as described before (Stegeman and Binder 1979), using a Cary 118-C recording spectrophotometer. Cytochrome content was estimated assuming extinction coefficients of 91 mmol/$L^{-1}cm^{-1}$ for cytochrome P-450, as previously determined by Omura and Sato (1964). Protein was determined according to Lowry et al. (1951) using bovine serum albumin as a standard. All analyses were performed at least in duplicate and usually triplicate on pooled samples.

Results — Sampling time was not a significant factor in the experiments. Three-way factorial analysis of variance (Sokal and Rohlf 1969) with treatment, tem-

TABLE 1. Activity and induction of hepatic microsomal (mic) enzymes in *Fundulus heteroclitus* acclimated to different temperatures. (Significantly different from 16.5°C control fish at $*P \leq 0.01$; $**P \leq 0.001$; $***P \leq 0.0001$.)

Character	16.5°C Control (5)[a]	16.5°C Treated (7)	6.5°C Control (6)	6.5°C Treated (8)	SE
Liver wt./body wt. (%)	4.0	4.2	3.5	3.5	0.2
mg mic. prot./g liver	6.3	6.3	5.9	6.6	0.2
Cytochrome P-450 nmol·mg mic. protein^{-1}	0.37	0.54**	0.38	0.42	0.04
NADH-cyt. c reductase[b] units·mg mic. protein^{-1}	498	518	511	442	27
NADPH-cyt. c reductase[b] units·mg mic. protein^{-1}	144	203***	181*	186	69
Aminopyrine demethylase[b] units·mg mic. protein^{-1}	153	183	185	178	69
Benzo[*a*]pyrene hydroxylase[b] units·mg mic. protein^{-1}	267	487***	360*	340	48

[a]Number of fish in two pooled samples. Data are ± standard error.

[b]Units are nanomoles cytochrome P-450/mg microsomal protein; nanomoles cytochrome c reduced·min^{-1} (reductases); nanomoles HCHO produced normalized to 1 h (AP demethylase); picomoles 3-OH-BP equivalents produced·min^{-1} (BP hydroxylase).

perature, and time as factors revealed that sampling time was not a contributing element in the main effects nor in two-way or three-way interactions. Accordingly, the data for 48- and 96-h samples at either temperature were combined for display purposes.

There were no significant differences in liver weight/body weight ratios or the yield of microsomal protein, between treated and control animals at 16.5°C. However, at this temperature there was a significant induction of benzo[*a*]pyrene hydroxylase activity upon treatment with the polynuclear aromatic hydrocarbon benzo[*a*]pyrene (Table 1). As suggested above this induction was evident at both 48 and 96 h posttreatment. The treated fish at this temperature also had significantly greater levels of NADPH-cytochrome c reductase activity. Cytochrome P-450 content was almost 50% greater in the treated fish, and the reduced, CO-bound Soret maximum was definitely lower in these than in the control animals, appearing closer to 449 nm rather than 450 nm. However, neither aminopyrine demethylase nor NADH-cytochrome c reductase were significantly different between the control and the treated animals at 16.5°C.

Like the fish at 16.5°C, those fish acclimated to 6.5°C showed little or no change in liver weight/body weight ratio or in yield of microsomal protein upon treatment with benzo[*a*]pyrene (Table 1). There were also no significant differences in NADH-cytochrome c reductase or aminopyrine demethylase activity. But, unlike the response of the warmer fish, there was no induction whatever of either benzo[*a*]pyrene hydroxylase or NADPH-cytochrome c reductase activity in the treated fish at 6.5°C. There was, nevertheless, a suggestion of increased cytochrome P-450 content in the treated fish, albeit modest.

The levels of both benzo[*a*]pyrene hydroxylase and NADPH-cytochrome c reductase activity were significantly greater in control fish acclimated to 6.5°C than in similar fish acclimated to 16.5°C (Table 1). The levels were 35 and 26% higher, respectively, in the colder fish. Aminopyrine demethylase activity showed a similar trend, being 21% higher in the 6.5°C animals, but the difference was not significant. Levels of NADH-cytochrome c reductase and cytochrome P-450 on the other hand differed by less than 3% between control groups of fish at the two temperatures.

These differences in basal activity of benzo[*a*]pyrene hydroxylase and NADPH-cytochrome c reductase between cold and warm fish were significant whether normalized to microsomal protein or to liver weight. But when normalized to body weight they were not, which presumably is a reflection of the greater liver weight/body weight ratios occurring in the warm-acclimated fish. In spite of this there was still a distinct trend to greater activity of benzo[*a*]pyrene hydroxylase in the 6.5°C fish when the activity was normalized to cytochrome P-450. This activity, a rough estimate of turnover number, was 1022 (±255) pmol·min^{-1}·nmol^{-1} P-450 in the 6.5°C animals, as compared to 718 (±111) pmol·min^{-1}·nmol^{-1} P-450 of those at 16.5°C.

The influence of 7,8-benzoflavone on benzo[*a*]pyrene hydroxylase activity was determined with microsomes from treated and control fish at both temperatures (Table 2). The percent inhibition at either 5×10^{-6} mol/L or 1×10^{-4} mol/L 7,8-benzoflavone differed only modestly between most groups, with all groups

TABLE 2. Influence of 7,8-benzoflavone in vitro on microsomal benzo[*a*]pyrene hydroxylase in induced and temperature-acclimated fish.

Sample	% Activity at 7,8-benzoflavone concentration		
	0	5×10^{-6} mol/L	1×10^{-4} mol/L
16.5°C: Control	100[a]	93 ± 11[b]	26 ± 2
Treated	100	88 ± 10	22 ± 4
6.5°C: Control	100	74 ± 13	26 ± 11
Treated	100	80 ± 8	27 ± 1

[a] 100% activities are like those in corresponding samples in Table 1.
[b] ± standard deviation.

inhibited between 70–80% at 1×10^{-4} mol/L. The higher flavone concentration inhibited activity in 16.5°C treated fish slightly more than in control fish at 48 h, but this was not apparent at 96 h.

Discussion — These results clearly show that temperature can strongly influence the induction of mixed-function oxygenases in fish, with a repression of the response at colder temperatures. The induction of hepatic benzo[*a*]pyrene hydroxylase by benzo[*a*]pyrene in warm-acclimated *Fundulus* occurred fairly rapidly, similar to induction observed in other fish treated with polynuclear aromatic hydrocarbons (Statham et al. 1978). The lack of simultaneous induction of aminopyrine demethylase suggests that the increased cytochrome P-450 content represents synthesis of a cytochrome P-450 catalytically distinct from those dominant in control animals, analogous to the situation seen in some mammals treated with polynuclear aromatic hydrocarbons. The fact that this induction was accompanied by a shift in the reduced, CO-bound Soret maximum further indicates that a new cytochrome was induced. This shift to the violet in cytochromes P-450 induced by treatment with polynuclear aromatic hydrocarbon-like inducers, long known in mammals (Alvares et al. 1967) has, heretofore, not been seen in intact microsomes of treated fish. It is, however, consistent with results of Elmamlouk et al. (1977) who were able to resolve a cytochrome P-450 with a Soret peak at 448 nm from little skate (*Raja erinacea*) treated with dibenzanthracene.

While the cold acclimated fish here showed no signs of induction, it is possible that such induction might eventually occur given continued, and extended, exposure. Yamauchi et al. (1975) observed, for example, that induction of hepatic glucose-6-phosphate dehydrogenase upon starvation and refeeding, a process occurring overnight in mammals (Weber and MacDonald 1960), was accomplished only after several weeks in trout acclimated to 5°C. Yet, we have found (Kuhnhold et al. 1979) that at 2–4°C winter flounder (*Pseudopleuronectes americanus*) exposed to 0.1 mg/L No. 2 fuel oil, which is a known inducer of fish MFO (Payne and Penrose 1975), showed no evidence of induction from exposure for periods as long as 63 d.

The finding the cold acclimated *Fundulus* had higher basal levels of mixed-function oxygenase activity in vitro than did warm fish agrees with the observations made by Dewaide (1970) using rainbow trout (*Salmo irideus*) and roach (*Leuciscus rutilus*). The results here on estimated turnover number further suggest this effect may be due to differences in the cytochromes P-450 at different temperatures. Dewaide (1970) noted, however, that in trout and roach aminopyrine demethylase and aniline hydroxylase activity levels were greater in cold animals even when normalized to body weight, opposite to our findings with *Fundulus*. The discrepancy lies in the fact that liver weight/body weight ratios we observed were higher in warm fish, while Dewaide observed the reverse, possibly due to restricted diet in that study. Fed to satiation fish at warmer temperature have much larger livers (Yamauchi et al. 1975). Thus, similar benzo[*a*]pyrene hydroxylase activity in cold and warm *Fundulus* when normalized to body weight, indicates that temperature may not really alter an animal's capacity for initial metabolism of aromatic hydrocarbons.

Fish at colder temperatures are clearly less responsive to inducers, and given similar xenobiotic exposure we might expect induction in warmer but not colder animals depending on the actual temperatures involved. Given the availability of sufficient food, an affect on induction accompanying even a slight difference in temperature could result in a greater capacity for hydrocarbon metabolism in the warmer animals. Such an affect may be contributing to the inverse relationship between acclimation temperature and retention of naphthalene per se in several tissues of coho salmon, noticed by Collier et al. (1978). A similar inverse relationship has been found in crustaceans by Harris et al. (1977), and while this might involve induction, there may as well be some other common affect of temperature on xenobiotic disposition. Temperature alters such features as lipid composition, which could influence rates and routes of hydrocarbon mobilization. This, or some other factor, could be controlling the rates of elimination. Apart from this, temperature could markedly affect induction in the environment, possibly influencing results of efforts, in colder seasons, to use fish mixed-function oxygenase activity as an environmental indicator.

Hepatic microsomal benzo[*a*]pyrene hydroxylase in the various treated and control groups of *Fundulus* could not be distinguished on the basis of response to 7,8-benzoflavone, which in some mammals preferentially inhibits the activity catalyzed by aromatic hydrocarbon-induced cytochromes P-450 (Wiebel et al. 1971). Others have also found benzo[*a*]pyrene hydroxylase in untreated fish to be strongly inhibited by 7,8-benzoflavone (Ahokas et al. 1975, 1977; Stegeman and Binder 1979) or 5,6-benzoflavone (Statham et al. 1978).

While on the one hand these data suggest that normal fish cytochromes P-450 may in some ways resemble those in aromatic hydrocarbon-treated mammals, Bend et al. (1977) and Stegeman and Chevion (1979) have in fact found that some other untreated fish have benzo[*a*]pyrene hydroxylase activity that is not inhibited by 7,8-benzoflavone. This supports the possibility (Stegeman and Binder 1979) that many ostensibly untreated fish, including control fish here, may have been partially induced by environmental chemicals.

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Recovery and Concentration of Infectious Pancreatic Necrosis (IPN) Virus in Water[1]

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GRINNELL, B. AND J. C. LEONG. 1979. Recovery and concentration of infectious pancreatic necrosis (IPN) virus in water. J. Fish. Res. Board Can. 36: 1405–1408.

Infectious pancreatic necrosis (IPN) virus was concentrated from water by a polymer two-phase method using polyethylene glycol and dextran sulfate. A concentration of over 100-fold was attained. By this method as little as one infectious virus particle per litre of water can be detected.

Key words: infectious pancreatic necrosis virus, virus concentration, diplornavirus, salmonids, polyethylene glycol

GRINNELL, B. AND J. C. LEONG. 1979. Recovery and concentration of infectious pancreatic necrosis (IPN) virus in water. J. Fish. Res. Board Can. 36: 1405–1408.

Les auteurs ont concentré le virus de la nécrose pancréatique infectieuse (IPN) par méthode polymérique biphasée utilisant le polyéthylène-glycol et le dextran sulfate. Ils ont atteint une concentration plus que centuplée. Cette méthode permet de déceler des concentrations aussi faibles qu'une particule de virus par litre d'eau.

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INFECTIOUS Pancreatic Necrosis (IPN), a viral disease of salmonid fish, typically attacks young fish, and usually high mortalities can result in severe economic loss for fish hatcheries. During a trout or salmon hatchery epizootic, large quantities of virus are released into the water. As much as 25 $TCID_{50}$ (50% tissue culture infectious dose) units per millilitre of water have been detected at the height of viral epizootics in hatcheries where water is flowing at a rate of 10–100 L/min (Wolf 1966). Some fish that survive these epizootics grow to adulthood and continually release virus with feces, eggs, and sperm (Billi and Wolf 1969). Thus, virus can be present in hatchery water from epizootics and from carrier fish because IPNV can persist for 8 wk in lake water without a significant loss in titer (Wedemeyer et al. 1978). As the disease can be transmitted by water directly (Wingfield and Chan 1970), an effective means for detecting very small amounts of virus in the water was needed. We have developed such a method and can detect as little as one plaque forming unit (PFU) per litre of water.

Concentration of IPN virus involves aqueous polymer two-phase separation of virus particles from liquid media (Albertsson 1958; Philipson et al. 1960). Such partitioning methods have been successfully used to concentrate small amounts of poliovirus from water (Shuval et al. 1967) and polio, coxsackie (Lund and Hedstrom 1967), ECHO and reoviruses (Nupen 1970) from wastewater and sewage (Grindrod and Oliver 1969).

Materials and methods — Cell cultures — The chinook salmon, *Oncorhynchus tshawytscha*, embryo cell line (CHSE-214) of Fryer et al. (1965) was used for all IPN virus assays and for the preparation of stock virus. The cells were grown at 18°C in plastic flasks (Falcon) in Eagle's minimum essential medium with Earle's salts (Autopow,

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Flow Lab) supplemented with 10% (vol/vol) fetal bovine serum, 0.002 M glutamine, penicillin (50 IU/mL) and streptomycin (50 μg/mL), (EMEM-10).

Standard virus — The IPN used in this study was the CTT strain isolated from cutthroat trout, *Salmo clarki*, obtained from the Oregon Game Commission. For preparation of IPNV stocks, CHSE-214 cells were infected at a low multiplicity of infection (MOI $\leq$ 1). Cells were incubated at 18°C until maximum cytopathic effect (CPE) was evident. The tissue culture fluid was then harvested and cell debris was removed by centrifugation. The virus-containing fluid was stored in 1-mL aliquots at −85°C.

Since IPN virus of the CTT serotype exhibits a marked decrease in titer upon freeze thawing (McMichael et al. 1975), the titer of the stock virus was determined for each experiment.

Infectivity assays — The virus titer for all samples was determined by the plaque assay. CHSE-214 cells were seeded onto 35-mm² plastic petri dishes (Falcon) in EMEM-10. Confluent cell monolayers were inoculated with 0.2 mL of the appropriate virus dilution. Virus was adsorbed for 45 min at 4°C with intermittent shaking of the dish. The infected cells were then overlayed with 0.6% agarose (Seakem) in EMEM-10 at 42°C. In some cases, when tap water was tested for virus recovery, the agarose overlay was supplemented with Mycostatin (30 units/mL). After 3–4 d incubation at 18°C, the cells were examined for plaque formation. The virus titer is given in plaque forming units per millilitre (PFU/mL).

Virus concentration — Test samples were prepared with different concentrations of virus in 0.5–1 L of sterile double-distilled water or tap water. The pH of each sample was adjusted to 6.5 with 5.0 mol/L NaOH. Polyethylene glycol (PEG) (mol. wt. 6000, Sigma Chemical) and dextran sulfate (DS) (average mol. wt. 500 000, Sigma Chemical) was added to a final concentration of 6.5 and 0.2%, respectively. Varying concentrations of NaCl were added in some experiments (see Results). The mixture was shaken in a separatory funnel for several minutes and the phases allowed to separate for approximately 24 h at 4°C. The lower, more dense DS phase and the interphase were removed together and for each gram of this phase, 0.55 mL of 3.8 mol/L KCL were added to precipitate the dextran sulfate. The KCL was added by drops to each sample with vigorous mixing between each addition. The mixture was then centrifuged at 1000 × *g* for 10 min in an HS-4 rotor, Dupont Sorvall RC-5 refrigerated centrifuge at 4°C. The supernatant was dialyzed against 100 mL of Dulbecco's phosphate buffered saline for every 2 mL of supernatant fluid for 2–4 h at 4°C. The dialyzed material was tested for viral infectivity.

Results and discussion — Albertsson (1960) reported that in polymer two-phase separation most viruses will be found in the dextran sulfate phase at 0.3 mol/L NaCl and in the PEG phase at 1.0 mol/L NaCl. This does not appear to be the case for IPN virus. Recovery of IPN virus increased dramatically with increasing concentration of sodium chloride (Table 1). Sodium chloride concentrations above 1.0 mol/L resulted in an emulsion that would not separate completely in 24 h. Therefore, all following experiments were conducted with 1.0 mol/L NaCl.

TABLE 1. Effect of NaCl concentration on IPN virus recovery in the DS phase.

NaCl concentration (mol/L)	% Recovery[a]	Concentration factor for virus[b]
0.3	2	3
0.5	14	23
1.0	64	119

[a]Recovery of input PFU in DS phase.
[b]Final concentration/initial concentration.

The test samples were prepared by seeding water with IPN virus at concentrations from 1.6×10^4 to 1.6×10^{-2} PFU/mL. These samples were then extracted by polymer two-phase separation as described. At all virus concentrations tested, we were able to attain an approximate 100-fold increase in virus concentration in the dextran sulfate phase (Table 2). Even at the lowest initial virus concentration of less than two infectious virus particles per 100 mL, virus was detected in the DS phase. Similar results were obtained repeatedly in different sets of experiments. Recovery of viral infectivity in the DS phase ranged from 53 to 71% of the total input (Table 3). When it was possible, the PEG phase was also examined for infectious virus. This information was used to determine the total viral infectivity recovered by this method (Table 3). Approximately 90% of the input virus was detectable in the DS and PEG phase. Since the dextran sulfate is precipitated with potassium chloride before that phase is assayed, the less than 100% recovery of total virus infectivity may be due to virus in the dextran sulfate precipitate. Another contributing factor in the loss of total virus infectivity may be the direct effect of the sulfated polysaccharide, dextran sulfate, which inhibits the attachment of some viruses to cells (Bengtsson 1965; Grindrod and Oliver 1969).

IPN virus can be concentrated from tissue culture fluid by precipitation with polyethylene glycol alone (Cohen et al. 1973); only a 10-fold concentration of the original material was obtained with 10% PEG. Tu et al. (1974) has successfully used the DS–PEG two-phase method for IPN virus concentration from tissue culture fluid. Their studies indicate that IPN virus can be concentrated at least 50-fold. The polymer two-phase method described here will concentrate IPN virus from water by more than 100-fold. This method is capable of concentrating large (several litres) amounts of water so that dilute virus levels can be detected. In all cases (Table 3), at least 50% of the input virus was detected in the DS phase. Thus, a minimum detectable virus concentration in water for this method is 1 PFU/L in a 2-L sample. Similar levels of virus detection by this method in water and wastewater have been reported (Hill et al. 1971; Nupen 1970; Shuval et al. 1969).

The polymer two-phase method is an economical, simple, and quantitative technique. Since IPNV is trans-

TABLE 2. Concentration of IPN virus from seeded water samples. Results are the average of duplicate runs at each initial concentration.

	Before concentration		After concentration		
Experiment	Vol. (mL)	Initial concentration (PFU/mL)	Vol.[a] (mL)	Final concentration (PFU/mL)	Concentration factor[b]
1	500	1.6×10^{4}	2.7	1.9×10^{6}	119
2	500	1.2×10^{4}	3.3	1.3×10^{6}	108
3	200	7.3×10^{0}	1.1	7.2×10^{2}	99
4	400	1.6×10^{-2}	2.1	1.9×10^{0}	119

[a]Volume of dialyzed dextran sulfate phase after precipitation of dextran sulfate with KCL (*see* Materials and Methods).
[b]Final concentration/initial concentration.

TABLE 3. Recovery of IPN infectivity after concentration

Experiment[a]	Total input (PFU)	Total virus in dextran sulfate phase (PFU)	Percent recovered in dextran sulfate phase	Total virus in PEG phase (PFU)	Percent recovered in PEG phase	Total IPN Infectivity recovered[b]
1	8.0×10^{6}	5.1×10^{6}	64	2.6×10^{6}	32	96
2	5.9×10^{6}	4.1×10^{6}	71	0.9×10^{6}	15	86
3	1.5×10^{3}	7.9×10^{2}	53	—	—	—
4	6.4×10^{0}	4.0×10^{0}	63	—	—	—

[a]Numbers correspond to those in Table 2.
[b]Total percent recovery was not determined for experiment 3 and 4 because of the low virus titer.

mitted by water, the release of IPNV in effluent water during hatchery epizootics may be undesirable for resident salmonid species. This method will be an effective tool for monitoring effluent water for IPN virus during epizootics. In addition it will be useful in evaluating IPN virus inactivation in water after treatment with ultraviolet light, chlorine, or ozone. An accurate measure of any remaining infectious virus can be made only after virus concentration. We believe that the polymer two-phase method will be an effective method for detecting IPN virus in hatchery water, and thus provide information for the control of IPN.

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Feeding by Hatchery-Reared and Wild Atlantic Salmon (*Salmo salar*) Parr in Streams

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Hatchery-reared Atlantic salmon (*Salmo salar*) parr were captured 1-3 mo after release in streams, along with wild parr from the same streams. Identification of their stomach contents showed total number of organisms and number of taxa per stomach were greater and there was a higher index of stomach fullness in wild than in hatchery parr resident $\leq$ 2 mo in a stream. Wild parr consumed more Brachycentridae, Hydroptilidae, Diptera, and Plecoptera than did hatchery parr, but sometimes less Odontoceridae and Heptageniidae. These differences may have arisen from size-dependent food selection, the effects of feeding experience, or possible microhabitat differences between wild and hatchery parr.

Key words: salmon parr, hatchery-reared, wild, feeding

SOSIAK, A. J., R. G. RANDALL, AND J. A. MCKENZIE. 1979. Feeding by hatchery-reared and wild Atlantic salmon (*Salmo salar*) parr in streams. J. Fish. Res. Board Can. 36: 1408–1412.

Des tacons de saumon atlantique (*Salmo salar*) élevés en pisciculture ont été capturés 1 à 3 mois après mise en liberté dans des cours d'eau, de même que des tacons sauvages dans les mêmes cours d'eau. L'examen de leurs contenus stomacaux démontre que le nombre total d'organismes et le nombre de taxons par estomac, ainsi que le bol alimentaire sont plus élevés chez les tacons sauvages que chez ceux d'élevage ayant résidé $\leq$ 2 mois dans un cours d'eau. Les tacons sauvages consomment plus de Brachycentridae, Hydroptilidae, Diptera et Plecoptera que les tacons d'élevage, mais parfois moins d'Odontoceridae et d'Heptageniidae. Il se peut que ces différences soient dues au choix d'une nourriture en fonction de la taille, aux effets de l'expérience passée en alimentation ou à des micro-habitats différents des tacons sauvages et des tacons d'élevage.

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IN many countries hatchery-reared salmonids are released in the wild to supplement reduced stocks. Hatchery-reared salmon smolts appear to contribute proportionately less to a fishery than do wild salmon (Österdahl 1969). If artificial feeding systems in a hatchery alter the feeding behavior of fish, they may forage less effectively when released in streams, than do wild fish of the same species. Miller (1951) found that 30% of age 3+ and 50% of age 2+ hatchery-reared cutthroat trout (*Salmo clarkii*) died during the first 40 d after release in streams, apparently from exhaustion and starvation. Hatchery cutthroat trout sampled by Hochachka (1961) had a lower weight of stomach contents 28 d after release than did wild trout from the same stream.

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Our study was conducted to determine differences in feeding between hatchery and wild Atlantic salmon (*Salmo salar*) that might reduce the survival rate of hatchery fish after release.

Materials and methods — This report presents the results of stomach content analyses on hatchery parr recaptured after residence in streams of the Northwest Miramichi River watershed and on wild parr from the same streams. Hatchery-reared parr were supplied by the South Esk Fish Culture Station of Environment Canada. They were age 1+ offspring of wild salmon; in 1976 they were taken from Northwest Miramichi and in 1977 from Bartholomew River salmon. Two days before release they were anaesthetized with tertiary-amyl alcohol and then individually marked using brass, cold-branding tools, similar to those of Everest and Edmundson (1967), that were cooled with liquid nitrogen and applied for 3 s. Salmon were weighed (nearest 0.1 g) and fork lengths measured (nearest millimetre) before release and after recapture (Table 1).

There were in total six releases of hatchery parr, with between 40 and 100 hatchery parr in each release (Table 1). Two releases were made on August 19, 1976, each in a different brook (Trout and Whitney brooks), and three releases were made the following spring (June 4), one in Whitney Brook and two in Trout Brook at sites about 4 km apart. The final release was made August 4, 1977 in Trout Brook and was paired with a simultaneous release of wild parr that were captured in a different brook and cold-branded 2 d earlier.

Each release area was electrofished between 27 and 95 d after release (Table 1) using a Dirigo 500 R, AC electrofisher. Each time a released parr was captured, one or more wild parr of similar size were electrofished if possible, from the same area. Captured parr were killed by concussion, and kept on ice during the collecting period. In 1976 each site was electrofished over a distance of at least 0.8 km upstream from the release area and 1.5 km downstream. As most hatchery-reared parr were found within 100 m of the release site, the length of stream electrofished was less in 1977. During a collecting period, each site was repeatedly electrofished until no more hatchery-reared parr were captured.

The stomachs of captured parr were removed and preserved in 10% formalin in 1976, and in AFA (90 parts 70% ethanol: 5 parts formalin: 5 parts acetic acid) in 1977. Later, cardiac and pyloric stomachs were opened separately and their fullness visually rated on a scale from 0.0 to a maximum of 2.0 in 0.5 increments. Cardiac and pyloric values were added to obtain an index of fullness for each stomach (Hynes 1950). Stomach contents were identified to family, if possible. Head capsules of partially digested insects were counted as single organisms. Terrestrial invertebrates and the adult forms of aquatic insects were grouped under the category "terrestrial and winged invertebrates."

The Mann-Whitney U-test was used to determine the two-tailed probability that wild and hatchery parr stomachs contained equal numbers of an organism.

Results and discussion — Wild parr consumed a greater total number of food organisms and had a higher mean index of fullness and greater number of taxa in five of the six collections (Table 2). The single

TABLE 1. Data on release and capture of Atlantic salmon parr; W, wild parr; RW, released wild; H, hatchery parr (SD in parentheses).

Stream and date of capture	Trout Bk. Site 1 June 30, 1977		Trout Bk. Site 1 Sept. 6, 1977			Whitney Bk. Oct. 1, 1976		Trout Bk. Site 2 Oct. 1, 1976		Whitney Bk. Aug. 4, 1977		Trout Bk. Site 2 Sept. 6, 1977	
	W	H	W	H	RW	W	H	W	H	W	H	W	H
Days since release		27		32	32		43		43		62		95
Number released		50		40	40		50		50		100		50
Number recaptured	9	13	19	20	9	20	18	18	6	19	17	18	4
Length at capture (mm)	107 (10.2)	104 (7.2)	111 (13.0)	124 (15.7)	118 (11.1)	117 (6.1)	146 (14.8)	120 (11.3)	142 (9.8)	109 (14.0)	106 (5.5)	112 (13.5)	121 (8.5)
Weight at capture (g)	15.2 (4.6)	12.9 (3.0)	14.9 (6.0)	21.4 (9.0)	17.1 (5.6)	18.0 (3.3)	32.4 (12.5)	19.5 (6.5)	30.8 (6.7)	15.6 (5.3)	13.0 (1.9)	17.1 (7.1)	19.9 (3.8)

TABLE 2. Mean number per stomach and per cent occurrence of food organisms in six collections of wild (W), hatchery (H), and released wild (RW) salmon parr. (Maximum number per stomach in parentheses).

	Trout Bk. Site 1 June 1977		Trout Bk. Site 1 Sept. 1977			Whitney Bk. Oct. 1976		Trout Bk. Site 2 Oct. 1976		Whitney Bk. Aug. 1977		Trout Bk. Site 2 Sept. 1977	
	W	H	W	H	RW	W	H	W	H	W	H	W	H
Simuliidae	106.1 (522)*[a]	5.5 (60)	0.1 (1)	0.2 (2)	—	—	—	—	—	5.2 (58)	0.7 (4)	0.1 (1)	—
	90%	38%	5%	10%						42%	18%	6%	
Chironomidae	6.2 (21)ns[a]	1.6 (16)	0.4 (3)	0.4 (2)	0.2 (1)	0.7 (7)	0.1 (1)	1.0 (4)*	—	12.6 (83)	4.7 (24)	1.1 (5)	0.5 (1)
	67%	38%	26%	35%	22%	15%	16%	50%		68%	76%	44%	50%
Other Diptera	4.8 (19)	5.3 (30)	0.9 (4)	0.5 (2)	0.8 (5)	0.9 (3)	0.3 (3)	0.1 (1)	—	2.7 (36)	0.6 (2)	1.0 (3)	0.5 (1)
	56%	46%	42%	45%	33%	55%	22%	6%		53%	47%	44%	50%
Hydroptilidae	0.1 (1)	—	—	—	0.2 (2)	8.7 (42) ns	6.7 (41)	1.3 (10)	0.2 (1)	0.3 (3)	0.1 (1)	—	—
	11%				11%	85%	44%	28%	17%	11%	12%		
Brachycentridae	0.2 (1)	—	—	—	—	3.9 (18)**	0.3 (5)	—	—	—	—	—	—
	11%					60%	15%						
Hydropsychidae	0.2 (1)	0.2 (1)	0.26 (3)	0.2 (1)	0.7 (4)	0.1 (1)	0.2 (1)	0.2 (1)	—	1.8 (14) ns	0.1 (1)	0.7 (6)	1.3 (2)
	22%	15%	16%	20%	33%	10%	17%	22%		37%	12%	28%	75%
Odontoceridae	—	—	0.1 (1)	—	—	0.2 (2)	0.3 (2)	0.2 (1)*	0.7 (1)	—	0.11 (1)	0.3 (3)	0.8 (2)
			5%			20%	28%	17%	67%		6%	11%	50%
Other Trichoptera	3.1 (11)	2.3 (5)	0.9 (3)	1.3 (5)	0.9 (2)	0.5 (2)	0.3 (3)	0.9 (3)	0.5 (2)	1.6 (18)	0.6 (2)	1.9 (9)	1.0 (2)
	67%	85%	47%	55%	56%	40% ns	17%	44%	17%	42%	47%	67%	50%
Heptageniidae	0.6 (2)	0.3 (2)	0.1 (1)	0.1 (1)	—	1.3 (4)*	2.6 (8)	—	—	0.4 (4)	0.2 (2)	1.0 (7)	0.8 (2)
	33%	22%	11%	5%		65%	83%			21%	12%	39%	50%
Baetidae	5.2 (16)	4.5 (47)	0.4 (2) ns	0.1 (1)	0.1 (1)	1.2 (3)	0.9 (4)	1.0 (4)	0.3 (1)	1.1 (8)	0.4 (2)	0.9 (3)	1.8 (4)
	78%	62%	26%	10%	11%	70%	44%	61%	33%	37%	35%	56%	75%
Plecoptera	0.7 (3)*	0.1 (1)	0.8 (3)	—	0.1 (1)	0.6 (2)	0.3 (3)	0.4 (4)	—	0.1 (1)	0.2 (2)	0.2 (2)	—
	44%	8%	47%		11%	40%	22%	28%		11%	18%	11%	
Other aquatic taxa	0.9 (2)	0.9 (4)	0.3 (2)	0.3 (2)	—	1.1 (15)	0.2 (2)	0.1 (1)	0.5 (2)	0.4 (2)	0.7 (4)	1.0 (2)	0.8 (2)
	67%	31%	21%	20%		20%	17%	6%	17%	32%	35%	28%	50%
Terrestrial and winged invertebrates	16.2 (66)	5.9 (19)	2.2 (16)	1.1 (4) ns	2.4 (7)	0.6 (5) ns	0.2 (2)	0.3 (3)*	1.3 (4)	4.8 (20)	6.4 (24)	2.7 (10)	4.3 (8)
	78%	77%	68%	60%	89%	40%	11%	17%	67%	79%	82%	88%	100%
Total number of food organisms per stomach	144.3 (574)*	26.7 (128)	6.4 (22)	4.0 (9)	5.4 (13)	19.7 (56)*	12.4 (56)	5.6 (15)	3.5 (7)	31.1 (119)	14.8 (36)	10.8 (25)	11.5 (16)
Index of fullness	2.9 (4.0)	2.2 (3.5)	2.2 (3.5)	1.9 (3)	2.2 (3.5)	2.4 (4.0)	1.9 (3.0)	2.0 (4.0)	1.8 (3.0)	2.5 (4.0)	2.1 (3.0)	2.8 (4.0)	3.0 (3.5)
Parasitic Nematoda[b]	5.8 (16)*	3.1 (26)	0.8 (3)**	0.1 (1)*	0.6 (2)	0.1 (1)	—	0.6 (2) ns	—	3.4 (9)	31.0 (21)	1.8 (7)	0.5 (1)
	90%	31%	47%	10%	44%	10%		44%		89%	76%	67%	50%

[a]Calculated probability: ns = $0.10 > P > 0.05$; * = $P < 0.05$; ** = $P < 0.01$.
[b]Not a food organism.

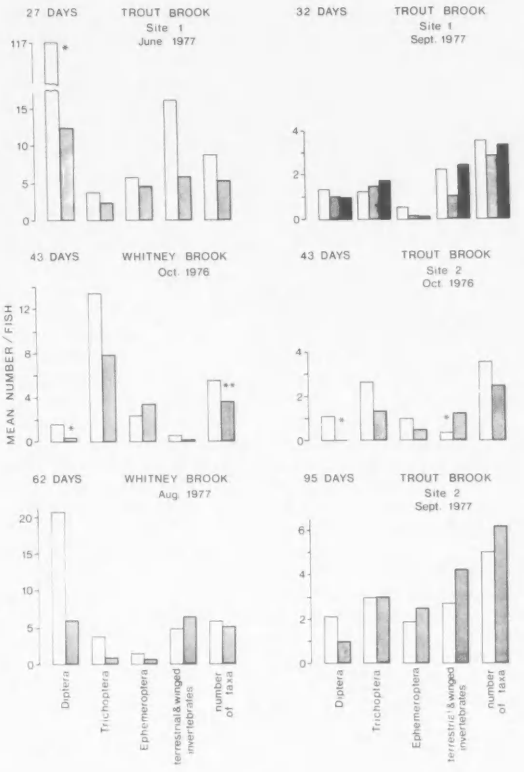

FIG. 1. Major taxanomic groups of food organisms present in the stomachs of wild (unshaded bars), hatchery (gray), and released wild parr (black). Times are days after release. Statistically significant differences are indicated (*= $P <$ 0.05; **= $P < 0.01$).

exception was only four hatchery parr that were resident in Trout Brook for 95 d before being collected.

In all six collections of hatchery and wild parr, the order Diptera was present in resident wild parr more often than in hatchery parr stomachs (Fig. 1), in half significantly more often ($P < 0.05$). Chironomidae was the most frequently consumed dipteran family, and Simuliidae was also consumed more by wild than by hatchery parr (Table 2).

The trichopteran families Hydroptilidae, Brachycentridae, and Hydropsychidae, and the order Plecoptera, were consumed more by wild than by hatchery parr in certain collections (Table 2). Hatchery parr, however, consumed significantly more of one caddisfly, Odontoceridae, than did wild parr in one collection and more Heptageniidae in another. Hatchery parr also took more terrestrial and winged invertebrates than did wild parr, after >6 wk stream residence, but less than did wild parr when resident <6 wk in a stream (Fig. 1).

The parasitic nematode *Sterliadochona tenuissima* was more prevalent ($P < 0.05$) in wild parr than in hatchery parr stomachs after about one month in a stream. This difference appeared to diminish with longer stream residence, perhaps through consumption of the ephemeropteran intermediate hosts (Hare and Frantsi 1974).

The fuller stomachs and wider food base utilized by wild parr could have resulted from: (a) size-dependent food selection, (b) the lack of appropriate "searching images" in hatchery parr and (c) microhabitat differences.

The size of a young salmon appears to influence its selection of food. Keenleyside (1967) found wild underyearling salmon consumed proportionately more Chironomidae than did 1+ and 2+ parr, and Wankowski (1977) found food particles 2.2–2.6% of fork length elicited the maximum feeding attack by hatchery parr. Size-dependent selection may have contributed to lower Diptera consumption in the present study where groups of hatchery parr were larger than wild parr. Size-dependent selection may also account for the greater consumption by hatchery parr of the large, cylindrical Odontoceridae.

Ware (1971) found that rainbow trout, *Salmo gairdneri*, acquire a "searching image," based on familiarity with a prey item, which facilitates future searches for that prey. Wild parr may possess a greater variety of prey searching images than do hatchery parr as a result of their more varied diet. This could explain the greater number of taxa identified in wild parr stomachs than in hatchery parr in this study.

In stream tank studies (Sosiak 1978), wild parr swam closer to the substratum and spent more time in direct contact with it than did hatchery parr. In the present study, the families Simuliidae, Brachycentridae, and Hydroptilidae, which all frequently cling to rocks while feeding, were consumed more by wild than by hatchery parr. Wild parr may have fed more on benthic invertebrates because of greater tendency to remain in contact with the substratum.

These results suggest that hatchery-reared parr feed less effectively than do wild parr, for at least 2 mo after release in streams.

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Difference in Taste Between the Right and Left Sides of Tainted Fish

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LIEM, A. J., AND V. A. NAISH. 1979. Difference in taste between the right and left sides of tainted fish. J. Fish. Res. Board Can. 36: 1412–1413.

More tainting was found on the left than on the right side of rainbow trout (*Salmo gairdneri*) that were exposed to a kraft pulp mill "foul condensate." This sidedness was deduced from the results of the triangle taste test used to detect differences between exposed and control fish, and was observed when the tainting was moderate, in the range of effluent concentrations of 0.015–0.046% by volume.

Key words: fish, tainting, taste, rainbow trout.

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On constate que le côté gauche de truites arc-en-ciel (*Salmo gairdneri*) exposées au «condensat impur» d'une usine de pâte à papier Kraft est plus gâté que le côté droit. Cette différence apparaît dans les résultats de l'expérience de dégustation utilisée pour déceler des différences entre poissons «exposés» et poissons «témoins», et on l'a observée chez des poissons modérément gâtés, sur une gamme de concentrations d'effluent de 0,015 à 0,046% en volume.

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ONE aspect of water pollution which lately has grown in prominence is fish tainting, the impairment of the taste of fish due to absorption of off-flavor causing substances. Recently, a study was carried out to develop standard procedures for fish tainting bioassays, including that for taste testing (Brouzes et al. 1978).

In this study, duplicate taste tests were conducted by using the left and right sides of the fish exposed to the tainting agent. This approach was used to minimize possible differences due to fish-to-fish variation. However, it then became apparent that, instead of obtaining duplication as originally intended, the left side was tainted more than the right side.

This note reports the taste test data that confirm the "sidedness" observation.

Materials and methods — The details of the bioassay procedures are described elsewhere by Brouzes et al. (1978); therefore, only an outline is described here. The tainting agent was a kraft pulp mill "foul condensate," an effluent stream containing reduced sulphur (mercaptans and sulphides), phenolic, and hydrocarbon compounds.

Rainbow trout (*Salmo gairdneri*) 20–25 cm long of both sexes were exposed to the diluted effluent at a series of different concentrations ranging from 0.0014 to 0.14% by volume, selected so that at the highest level tainting could

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TABLE 1. Replicate triangle test results showing a difference in tainting between the right and left sides of fish by proportion of correct response.

Side of fish	Time of testing		Day of testing		Total
	AM	PM	First	Second	
Left	18/30	20/30	20/30	18/30	38/60
Right	8/30	8/25	8/30	8/25	16/55
Total	26/60	28/55	28/60	26/55	

TABLE 2. Triangle test results showing the effect of effluent concentration on the difference in tainting between the left and right sides by proportion of correct response.

Effluent concentration (% by volume)	Side of fish	
	Left	Right
0 –0.014	101/340	87/339
0.015–0.046	166/250	135/264
0.052–0.138	120/130	126/135

be detected but not at the lowest level. The fish were removed from the test solutions, beheaded, gutted, washed, and then split down the back bone. The two halves were wrapped in aluminum foil and frozen at −10°C until they were cooked and used in the taste test.

At each concentration level, the triangle test was used to determine whether or not the fish was tainted, that is, whether or not a difference in taste could be detected between the exposed and the control fish. Three samples — consisting of either two exposed and one control or two control and one exposed — were presented to each panelist who had to select and identify the odd sample.

The proportion of correct response in a series of triangle tests were taken as a measure of degree of tainting, and differences were analyzed with a chi-square test.

The sidedness effect was first noted towards the end of the aforementioned study in a series of tests carried out to determine the effect of fatigue on taste testing. This series of tests consisted of four sessions of replicate taste tests conducted over 2 d, in the morning and afternoon of each day. The right side of the fish was used in the morning session of the first day and the left in the afternoon. This order was then reversed for the second day of testing. Six panelists participated, with each panelist taking five triangle tests in each session. (One panelist was absent in the afternoon session of the second day.)

Results and discussion — Table 1 shows the proportion of correct response given in each session of the replicate tests. More tainting was found on the left than on the right side (chi-square test, $P < 0.01$), whereas neither the time of day nor the day of testing was a factor.

After this somewhat unexpected observation was noted, all the taste test results previously obtained were reexamined to determine whether the sidedness effect had in fact been obtained. Comparison between the right and left side results was made at each effluent concentration under each different exposure condition used in the aforementioned study. The results for each comparison were then pooled and grouped into three ranges of effluent concentrations.

Table 2 shows the proportion of correct response given in the three selected ranges of effluent concentrations. In the intermediate range, but not outside this range, more tainting was found on the left than on the right side (chi-squared test, $P < 0.01$).

Perhaps this concentration effect is not so surprising. When the tainting is low the difference between the exposed and the control fish is so slight that the difference between the right and left sides is hardly detectable. And when the tainting is very high, the difference between the exposed and the control fish is so obvious, that the selection and the identification of the odd sample in the triangle test would no longer depend on the degree of tainting.

The sidedness effect was quite unexpected and, to our knowledge, is biologically inexplicable. We carefully scrutinized the experimental procedures and are satisfied that they were not the source of the curious results reported. Publication of this sidedness observation should therefore be of interest to those conducting fish tainting bioassays and those studying the mechanisms for absorption of tainting substances in fish.

Acknowledgments — This work was part of a study sponsored by CPAR (Cooperative Pollution Abatement Research) grant no. 775 entitled "Protocol for Fish Tainting Bioassays."

BROUZES, R. J. P., A. J. LIEM, AND V. A. NAISH. 1978. Protocol for fish tainting bioassays. Fisheries and Environment Canada, Environmental Protection Service, CPAR Rep. No. 775.

Fluoride in Antarctic Krill (*Euphausia superba*) and Atlantic Krill (*Meganyctiphanes norvegica*)

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SOEVIK, T., AND O. R. BRAEKKAN. 1979. Fluoride in Antarctic krill (*Euphausia superba*) and Atlantic krill (*Meganyctiphanes norvegica*). J. Fish. Res. Board Can. 36: 1414–1416.

Samples of Antarctic krill (*Euphausia superba*) and Atlantic krill (*Meganyctiphanes norvegica*) were analysed for fluoride using a selective fluoride electrode method. Both species showed very high concentrations, a total of 1330–2400 mg F^-/kg on fat free dry weight basis in raw samples whereas deep sea prawns (*Pandalus borealis*) showed a total of 18–91 and red feed (*Calanus finmarchicus*) 10–37 mg F^-/kg. Sections of Antarctic krill were analyzed, and the highest concentration was found in the carapace, 4260 mg F^-/kg fat free dry matter, compared with 570 and 750 mg F^-/kg in raw and boiled muscle. Biological availability was measured in a chick assay, and showed fluoride in krill to be equally utilized as sodium fluoride added to the diet.

Key words: krill, red feed, deep sea prawn, fluoride

SOEVIK, T., AND O. R. BRAEKKAN. 1979. Fluoride in Antarctic krill (*Euphausia superba*) and Atlantic krill (*Meganyctiphanes norvegica*). J. Fish. Res. Board Can. 36: 1414–1416.

La teneur en fluorure d'échantillons de krill antarctique (*Euphausia superba*) et de krill atlantique (*Meganyctiphanes norvegica*) a été déterminée suivant une méthode faisant appel à une électrode sélective de fluorure. Les deux espèces contiennent de fortes concentrations, un total de 1330 à 2400 mg F^-/kg de poids sec sans gras dans des échantillons crus, alors que la crevette de profondeur (*Pandalus borealis*) en renferme de 18 à 91, et le copépode (*Calanus finmarchicus*) de 10 à 37 mg F^-/kg. Nous avons analysé des sections de krill antarctique et trouvé le maximum de concentration dans la carapace, 4260 mg F^-/kg de matière sèche libre de gras, comparativement à 570 et 750 mg F^-/kg dans le muscle cru et bouilli. Nous avons déterminé la disponibilité biologique dans des essais avec poulets et démontrons que le fluorure présent dans le krill est utilisé au même degré que le fluorure de sodium ajouté au régime alimentaire.

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THROUGH the years, data have accumulated on the occurrence of fluoride in different foods (Waldbott 1963). These studies have established tea and seafood as major sources. These foods make an important contribution of fluoride to our diet, where the requirement is considered to be of the order of 2.5 mg F^-/kg to ensure optimal growth and normal incisor pigmentation (Schwarz 1974).

With the introduction of the selective fluoride electrode, rapid and more precise determination of this element was made possible. To make available up to date values for the occurrence of fluoride in fish and fishery products, an extensive study is in progress at our institute. Among the samples analyzed were Antarctic krill, *Euphausia superba*, and the largest krill in Norwegian coastal water, *Meganyctiphanes norvegica*. Both species showed very high concentrations of fluoride. As Antarctic krill in its great abundance is viewed as a potential contribution to our food resources, the finding called for the studies of anatomical distribution and biological availability reported below.

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Materials and methods — Raw deep-frozen samples were brought to the laboratory. The Antarctic krill represented a mixture of many catches. The samples were divided into two lots. One was freeze dried and the lipid extracted with diethyl ether in a Soxhlet apparatus. The other lot was cooked in twice its weight of distilled water. After 10 min the water was discarded, and the solid was freeze dried and lipids extracted. The dried samples were homogenized and 0.1–0.2 g wet digested in Sovirel test tubes with 4 mL of a mixture of concentrated nitric acid + 70% perchloric acid (1:1 v/v). (Julshamn and Braekkan 1975). After cooking the digest was diluted to a volume of 8 mL with redistilled deionized water. The liberated fluoride was extracted into 4 mL diphenylsilanediol in toluene (0.2% w/v), followed by back extraction into 4-mL 10 N KOH. The pH was adjusted to 5.3 and the fluoride concentration measured by a selective fluoride electrode. The procedure was a modification of the method of Venkateswarlu (1974), with higher concentrations in the final solution, bringing the measurements into the linear range of the standard curve.

As a part of a nutritional evaluation of krill, the biological

TABLE 1. Fluoride (mg/kg) in fat free dry matter from samples of krill, red feed, and deep sea prawn.

Sample of	No.	Treatment	Whole	Sections				
				Muscle	Exoskeleton	Carapace	Cephalothorax	Pedipalps
Krill								
Euphausia superba	1	Raw	2400	570	3330	4260	3690	1950
		Boiled	2310	750	3000			
Meganyctiphanes norvegica	1	Raw	1330					
	2	Raw	2360					
		Boled	1680					
	3	Boiled	1260					
	4	Boiled	2870					
Red feed								
Calanus finmarchicus	1	Raw	13					
		Boiled	13					
	2	Raw	10					
		Boiled	15					
	3	Raw	35					
		Boiled	37					
Deep sea prawn								
Pandulus borealis	1	Raw	18	0.5		36	32	
	2	Raw	72	1.8		156	150	
	3	Raw	91	2.2		169	148	
	4–9	Boiled			260–570			

availability of the fluoride had to be determined. Zipkin et al. (1970) found the availability of fluoride in FPC to female rats to be about 25–50% that of sodium fluoride, whereas Spencer et al. (1970) found nearly equal availability of fluoride from the same sources in their study on adult humans. Chicks have been found to have a particularly high tolerance to fluoride (Underwood 1971), and an assay was developed using male White Leghorn. In this species, linearity in response could be observed with doses up to 500 mg F^-/kg diet. Day old chicks were grouped and fed a basal diet very poor in fluoride with graded additions of sodium fluoride and of krill, adjusted to the same fluoride levels. The response was measured in individual chicks in each group as the total fluoride concentration in the bones of the third toe. The availability of fluoride was calculated as the ratio between responses. A complete description of the method, which showed high reproducibility in repeated assays, will be published elsewhere.

Results and discussion — Table 1 summarizes results of analyses of Antarctic krill (*E. superba*), whole and sectioned, and raw and boiled samples of the krill *M. norvegica*. For comparison the crustaceans' red feed (*Calanus finmarchicus*) and deep sea prawn (*Pandalus borealis*) have been included, the latter also sectioned. The two species of krill showed overall F^- content in the raw samples to range between 1330–2400 mg/kg and for the boiled samples between 1260–2870 mg F^-/kg. When the Antarctic krill was sectioned, the meat contained as high as 570 mg F^-/kg in the raw and 750 mg F^-/kg in the boiled samples. Fluoride was strongly concentrated in the hard tissues, with the highest level in the carapace (4260 mg F^-/kg). Red feed showed low total contents, of the order of 10–35 mg F^-/kg for raw and boiled samples. For deep sea prawn the raw muscle samples showed very moderate values, 0.5–2.2 mg F^-/kg or of the order of data reported for fish by Souci et al. (1969), whereas the highest contents were found in six samples of the exoskeleton or total peel from processing plants, with 260–570 mg F^-/kg.

Analysis of krill samples by SINTEF (D. Fremstad, Central Institute for Technical Research, Trondheim,

TABLE 2. Fluoride concentrations in fat-free dry matter from toe bones of young chicks fed different levels of NaF and freeze dried krill.

Dietary F^- supplement (mg/kg diet)	0	1	2	4	8
Groups fed:		μg F^-/g toe bone ± SD (n = 5)			
NaF	11.6±3.1	28.6±5.3	36.8±9.5	70.0± 9.2	117.0±20.6
Krill[a]		25.2±5.4	35.0±2.4	64.6±11.9	102.6±11.1
Difference[b]		N.S.	N.S.	N.S.	N.S.

[a]Total content of 1260 mg F^-/kg.
[b]Significant level of difference between NaF- and krill-fed groups. Not significant at $P > 0.05$.

personal communication) and of similar samples by NMFS laboratories (J. Brooker, NMFS, U.S. Department of Commerce, 3300 Whitehaven St. N.W., Washington, D.C., 20235, personal communication) have yielded similar results.

Table 2 summarizes the results from assays of the availability to chicks of fluoride in whole krill compared to that of sodium fluoride. The response was linear in relation to supplementation, and at all fluoride levels there were no significant difference between retention of F^- from either source by groups of five chicks ($P > 0.05$). From this result it can be concluded that fluoride in krill is nutritionally readily available.

It has long been recognized that teleosts show a strong concentration of fluoride in the hard tissues, in particular the skeleton. When fish protein concentrate (FPC) from whole fish was first introduced for human consumption, the possible presence of high concentrations of fluoride in the product was considered. In the USA, the Food and Drug Administration (Anon. 1967) set up an upper permissible limit of 100 mg/kg calculated as sodium fluoride. The present values for fluoride in krill exceed this limit by more than seven times for the freeze dried and extracted meat, and 24 times for the entire shellfish. This would make krill in any form, even peeled, fail to comply with requirements for human consumption.

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Comment on
Management Alternatives and Strategic Planning for Canada's Fisheries

COMMENT

In a recent perspective it was claimed that using a tax on landings as a management tool in fisheries would not result in any serious administrative problems (Sinclair 1978). To see that this is not the case one need only consider the following model of taxation in a fishery (Anderson 1977).

Equilibrium in an unregulated fishery occurs where the average revenue per unit of effort equals the marginal cost:

$$(1)\quad \frac{PQ}{E} - C = 0$$

where P is the price of fish, Q is landings, E is effort, and C is marginal cost. This is a zero profit position with all of the resource rent dissipated. The economic optimum is reached where the marginal revenue per unit of effort is equal to the marginal cost:

$$(2)\quad P \cdot \frac{\Delta Q}{\Delta E} - C = 0$$

where Δ represents a small change. In order for a tax to induce fishermen collectively to arrive at this point it must be such that average revenue after tax is equal to marginal revenue.

$$(3)\quad \frac{PQ}{E} - Te = P\frac{\Delta Q}{\Delta E}$$

where Te is a tax per unit of effort. When solved for Te, this results in:

$$(4)\quad Te = P\left(\frac{Q}{E} - \frac{\Delta Q}{\Delta E}\right)$$

where P, $\frac{Q}{E}$, and $\frac{\Delta Q}{\Delta E}$ are those which would occur at the optimum harvest level. This can be converted to a tax on landings by multiplying the tax on effort by the average number of units of effort per unit of output:

$$(5)\quad Tq = Te \cdot \frac{E}{Q} = P\left(1 - \frac{\Delta Q}{\Delta E} \cdot \frac{E}{Q}\right)$$

where Tq is a tax per unit of output. It can be seen from (5) that the proper tax for steady state management is a function of the price of fish, and the average and marginal productivities of effort at the optimum harvest level. In a static world these would need to be evaluated only once but in the real world of changing economic conditions the optimum level of output would not be static. Any changes in demand for the fish product, cost conditions in the fishing industry, or in the growth pattern of the stock would change the economic optimum and would mean that a new tax would have to be calculated. It is also assumed that all fishermen fish on grounds of equal productivity and the tax will not achieve efficient allocation of resources if there are grounds of varying productivity. Finally, if a dynamic optimum is considered where fishermen respond to the net present value of the fish stock rather than the current value only the complexity of the tax increases.

It was also stated that the average earnings of fishermen should increase substantially in the long run, following the introduction of a tax. However, for the tax to remain as an effective regulatory device earnings must not rise above opportunity cost as this would induce an increase in effort. Earnings by fishermen both before and after regulation must, in equilibrium, be the zero profit position characteristic of a competitive industry. The difference is that the resource rent is captured through the tax rather than being dissipated. Individual fishermen can only be better off if the tax revenues are distributed back to the industry in a manner independent of the amount of effort applied by individuals.

Finally, as a comment on the acceptability of such a tax, it can be calculated from results obtained in a study of the United States northern lobster industry that a tax of 30% would be necessary to achieve the economic optimum (Bell 1972). While it is impossible to generalize from this one example, it may well be that tax rates necessary to achieve optimality would be politically unacceptable.

While taxation can be used to achieve optimality in a fishery the practical problems of implementation and administration necessary to ensure that the tax is the correct one will undoubtedly place a considerable burden of information gathering and monitoring on the agency in charge of this policy. — RICHARD L. MCGAW, *Department of Economics, University of New Brunswick, Fredericton, N.B. E3B 5A3*

ANDERSON, L. G. 1977. The economics of fisheries management. John Hopkins University Press, Baltimore, Md. 214 p.

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REPLY

The comment provided by Dr McGaw on my management alternatives paper is both interesting and educational. I share with him many of the concerns outlined in his comment. Nonetheless, I have a number of misgivings about McGaw's interpretation of what was said in my paper. Moreover, I feel that most of his stated concerns serve to focus on problems that might be encountered in particular fisheries if a tax on landings system was introduced. The concerns do not, as implied in his comment, detract from the management system advocated in my paper.

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It is important to recognize that the emphasis in my paper was to develop a management system that could be applied to all of Canada's fisheries. The recommended system was developed on the basis that it would contribute to the attainment of five different sets of stated criteria, all of which were identified (with some qualifications) as being consistent with economic efficiency. In contrast McGaw takes the view that the recommended management system was intended solely for the purpose of achieving economic optimality. All of his concerns center on problems associated with introducing a tax on landings; he ignores the positive influence that other aspects of the recommended program (license limitation and the grandfather scheme) would have on fisheries management. In my view, he takes the overly rigid prospective that a tax on landings system cannot be administered in a manner that contributes to economic efficiency unless the tax captures all of the resource rent. From this position he argues that it would be overly difficult to measure the exact level of the tax and the tax would be so great as to be politically unacceptable.

McGaw's comment contains three specific criticisms. The first is that a tax on landings would pose serious administrative problems because of complexities that would arise from introducing a tax system. The main thrust of this remark is directed at the assertion in my paper, that the adoption of a tax on landings would not pose any serious administrative problems because fish landings are always weighed and counted. The point of this remark was to draw a distinction between a tax on landings and other management alternatives described in the paper. It was not suggested that the implementation of a landings tax could be introduced without the appropriate administrative mechanisms; nor was it suggested that these administrative mechanisms could be adopted without considerable care. What was suggested, however, is that the tax on landings be slowly introduced, carefully monitored, and frequently adjusted. It also was suggested that the system be flexible to take account of the differences encountered among different jurisdictions.

The second criticism is that the average earnings of fishermen would not increase. McGaw asserts that for the tax to remain an effective regulatory device earnings must not rise above opportunity cost as this would induce an increase in effort. Aside from the fact that McGaw is ignoring the influence of license limitation, his statement implies that improved efficiency in the fishing industry would not increase the available economic returns. If a tax on landings would help to improve economic efficiency, then it follows that the economic returns available within the industry would increase. It would mean that fishermen, the processing industry, and the owners of the resource (the Canadian public) could expect to share greater returns because of improved efficiency. There is absolutely no reason to expect that one segment of the industry would take a greater portion of the total economic rent after the implementation of a tax than was the situation before establishment of a tax. The only difference would be that some of the resource rent would be captured on behalf of the public rather than be dissipated.

The third criticism is that the magnitude of the tax necessary to achieve an economic optimum would be so large that it would be politically unacceptable. This follows from McGaw's personal view that the tax on landings must capture the full amount of the resource rent and his implied belief that the full amount of the tax must be applied immediately. Even if one accepts that the full amount of the tax has to be taken, it does not follow that it would be politically unacceptable to capture the resource rent through a method of taxation. The resource rent is not captured by fishermen at present, nor is it captured by the processing industry. It is the failure to capture that portion of the resource rent dissipated through over exploitation, overcapitalization, and fishing restrictions that leads to major management problems in commercial fisheries. Once the fishermen, the industry and the public understand that the tax is aimed at that portion of the total potential economic returns that are presently dissipated at a great deal of expense to the fishermen, the fisheries resource, and to all Canadians, the introduction of a tax will not be so politically impalatable.

McGaw is, of course, correct in his contention that a great deal of care has to be taken when establishing a management system that encompasses a mechanism for capturing some resource rent. He sets forth many of the arguments and concerns that would be voiced if such a system were adopted. However, he fails to appreciate that economic optimality is not necessarily good in itself. It is the many advantages that would result from better economic performance that is important. There has been a tremendous amount of empirical evidence produced during the past 20 years that shows the economic and social hardships created by poor economic performance in the primary fishing industry. Any system that would improve economic performance would help to alleviate some of these hardships. The question is not whether or not a new management system would result in some ideal solution to all fisheries problems, but whether it would work well enough to improve the welfare of those involved in the industry and our ability to handle our nation's fisheries resources. — WILLIAM F. SINCLAIR, *Department of Environment, Office of the Regional Director General, Vancouver, B.C. V6E 2M7*

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Freshwater Fishes of Canada

W. B. SCOTT AND E. J. CROSSMAN

For many years there was an acute need for a publication on the identification, distribution, biology, and economic importance of the freshwater fishes of Canada. In 1973, Bulletin 184 of the Fisheries Research Board entitled *Freshwater Fishes of Canada* was published. A French translation entitled *Poissons d'eau douce du Canada* appeared in 1974. A separate Errata sheet providing corrections to the first English edition was circulated in 1975 and those corrections were incorporated in 1975 English and French reprints. The French edition was reprinted without further change in 1978.

The need in 1978 to reprint the English edition provided an opportunity for further improvements. The 1979 reprint of the English Edition is not a revision. It does, however, incorporate corrections not available earlier, and in addition includes a new introductory section. That section provides reference to 11 species new to the fauna, not included in the earlier editions, clarification of problems discovered in earlier printings, and a limited and selected amount of new information on some species.

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Relationship of cadmium-induced hypocalcemia with mortality in rainbow trout (*Salmo gairdneri*) and the influence of temperature on toxicity **M. Roch and E. J. Maly** 1297–1303

Bioeconomic model of the Gulf of Carpentaria prawn fishery **C. W. Clark and G. P. Kirkwood** 1304–1312

Overdominance of a muscle protein (*Mp*-1) locus in the Japanese oyster, *Crassostrea gigas* (Ostreidae) **N. E. Buroker** 1313–1318

Carcinonemertes errans and the fouling and mortality of eggs of the Dungeness crab, *Cancer magister* **D. E. Wickham** 1319–1324

Le contrôle de la variabilité à court terme du phytoplancton estuarien: stabilité verticale et profondeur critique **L. Fortier et L. Legendre** 1325–1335

Examination of phosphorus cycling and control of phytoplankton dynamics in Lake Ontario with an ecological model **D. Scavia** 1336–1346

Temperature and photoperiod effects on gill (Na + K)–ATPase activity in chinook salmon (*Oncorhynchus tshawytscha*) **R. D. Ewing, S. L. Johnson, H. J. Pribble, and J. A. Lichatowich** 1347–1353

Body size and feeding ecology as alternatives to taxonomy for the study of limnetic zooplankton community structure **W. G. Sprules and L. B. Holtby** 1354–1363

Indicators of phosphorus and nitrogen deficiency in five algae in culture **F. P. Healey and L. L. Hendzel** 1364–1369

Bacterial kidney disease in feral populations of brook trout (*Salvelinus fontinalis*), brown trout (*Salmo trutta*), and rainbow trout (*Salmo gairdneri*) **D. L. Mitchum, L. E. Sherman, and G. T. Baxter** 1370–1376

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Notes

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Feeding by hatchery-reared and wild Atlantic salmon (*Salmo salar*) parr in streams **A. J. Sosiak, R. G. Randall, and J. A. McKenzie** 1408–1412

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Letters/Lettres: Comment on management alternatives and strategic planning for Canada's fisheries **R. L. McGaw**; Reply **W. F. Sinclair** 1417–1418

Coming next month/À paraître le mois prochain 1419

Recent publications/Récentes publications 1420–1421

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